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Detection of Unknown Emerging Contaminants in Natural Water Using Tandem Mass Spectrometry

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DETECTION OF UNKNOWN EMERGING CONTAMINANTS IN NATURAL WATER USING TANDEM MASS SPECTROMETRY

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ABSTRACT

Recently, a great deal of concern has been given to the quality of drinking water. Lake Michigan is the drinking water source of Chicago area. It is very important that the quality of drinking water meets the standard setup by the EPA. However, there is a group of chemicals that are not being monitored regularly or are unknown to researchers and are the so-called emerging contaminants.

So the objective of this study is to using tandem-mass spectrometry to detect and identify emerging contaminants. Water samples were obtained along a few locations along the Chicago River and Lake Michigan. By using the precursor ion of chlorine, those ions that contain chlorine were selected for further study. The accurate masses of selected precursor ions were obtained using the quadrupole-time-of-flight (QTOF). By searching the online databases, empirical formulas were tentatively assigned. Product ion spectrum and other research methods including synthesizing standards are utilized to assign the chemical structure unambiguously.

3,5-Dichloro-4-hydroxy-benzenesulfonic acid is the first unknown identified by this method. Further ions will be identified by the same research method.

In a summary, tandem mass spectrometry coupled with high resolution mass spectrometry and online database searching is an effective method to identify known-unknowns. After the identification of these emerging contaminants, remedies should be proposed to degrade these contaminants.

CHAPTER ONE

EMERGING CONTAMIMANTS IN NATURAL WATER

Need for Water Purification

Recently, the quality of drinking water has become a concern due to emerging novel micropollutants. The novel micropollutants include pharmaceuticals and personal care products (PPCPs), water disinfection byproducts (DBPs), and flame retardants among other compounds [1]. These chemicals could be discharged into natural aquatic ecosystem without satisfactory treatment. The presence of these micropollutants in water sources could result in diseases such as cholera, dysentery particularly in countries where no sufficient water treatment facilities are available. Pathogens such as E. coli and Salmonella species are associated with adverse effects to humans [2]. It is essential to maintain a safe drinking water supply and a healthy aquatic habitat/environment. Therefore, water purification process is necessary. About one half of the US population relies on ground water as drinking water source, so ground water contamination could result in serious public health problems. Sources of ground water contamination includes subsurface disposal of domestic waste water through septic tank absorption fields. Non reclaimable industrial waste water and sludges are discharged to basins, pits and buried in landfills. Possible leakages of those hazardous substances and percolation of stored waste water into the ground are sources of pollution as well.

Other US population relies on surface water (rivers, lakes, and reservoirs) as drinking water source. Surface water is also vulnerable to contamination by animal waste, pesticide

contamination, and other potentially unknown sources. Pollutants in animal waste can enter the surface water through direct discharges to surface waters, while pesticides contaminants are drained into surface water systems by agricultural and urban area irrigation. Animal waste can also be transported over the surface of farm land to nearby lakes and streams. The major ecological impact associated with animal pollutants in surface waters are fish kills. Pollutants in animal waste could also affect human health. With the awareness of the adverse effects of the pesticide DDT, the problem of pesticides in surface water has received great attention during the last few decades. Additional pollutants result from industrial wastes. If these pollutants escape the waste water treatment process, the drinking water supply will be unsafe.

Brief History of Water Purification

The earliest water treatments in order to improve the taste and odor of drinking water dates back to 4000 B.C. [3]. At that time, treatment methods included filtering through charcoal, exposing to sunlight, and boiling. In 1500 B.C., Egyptians started using the aluminum sulfate to precipitate suspended particles to settle out of water. Later, during the 1700s, filtration was found as an effective means to remove particulate matter. In the late 1880's, scientists gained knowledge of how microscopic organisms could transmit diseases (typhoid, dysentery, and cholera) through water. Concerns regarding disease-causing pathogens in drinking water drove some water systems in U.S. begin to use slow sand filtration.

Although filtration is effective to reduce turbidity, the role of disinfectants such as chlorine played in reducing the number of waterborne disease outbreaks in the early 1990s is essential. In fact, chlorine was first used as a primary disinfectant of drinking water in 1908. Besides chlorine, other disinfectants, ozone and chloramine are used as well for disinfection.

Waste Water Treatment Process

Waste water treatment process involves the following steps: first, filter out the particle matter; second, use microorganisms to degrade organic matter; third, use chlorine to oxidize organic matter. The disinfection byproducts (DBPs) are usually generated at the third step of waste water treatment when chlorine is used as the oxidizing reagent. Except for DBPs, heavy metals are often found present in waste water from industrialized cities.

The first step of waste water treatment involves removal of particulate matter. The main method of removing particulate matter is coagulation. The major chemical reagents used are aluminum sulfate, whose common name is alum; calcium hydroxide, whose common name is lime. Ferric sulfate and ferric chloride are also used. Alum is usually used for drinking water treatment, whereas the iron compounds are used predominantly in domestic and industrial waste water treatment. The purpose of coagulation is to aggregate smaller particles into larger sizes that will settle down within one or two hours. Some bacteria and algae could be removed partially by coagulation. Natural organic matter (NOM), as the precursors for the formation of disinfection byproducts via the reactions with chlorine, could also be removed by coagulation. Generally speaking, coagulation reduces the amount of oxidizing agents needed later in the treatment process since part of the NOM is removed by coagulation.

Another method of removal of particulate matter is filtration and sedimentation.

Filtration is widely used in water treatment to remove solids; silica sand and garnet are the most commonly used materials in granular bed filters. Garnet is a polysilicate mineral. Types of filters include slow sand filters and rapid sand filters. A slow sand filter consists of a basin containing a layer of sand with the thickness of 3-5 ft over a 0.5-1 ft gravel. A rapid sand filter

consists of a 2 to 2.5 ft sand layer over a layer of gravel. Slow sand filters are very effective in the removal of microorganisms such as bacteria, salmonella, coliforms, virus, organic and inorganic contaminants, especially in the removal of Giardia cysts [4]. Giardia cysts are responsible for Giardiasis, whose symptoms include loss of appetite, diarrhea, stomach cramps, and vomiting. In the outbreak of Giardiasis in several areas of US, including Colorado, New Hampshire, Washington and New York, the most serious case occurred in a small mountain town of Empire, Colorado, and as many as 110 cases of waterborne giardiasis were reported in 1981, where chlorination was the only treatment process. With the usage of sand filter in 1985, no Giardia cysts were detected in the effluent.

The next step of waste water treatment is chlorination. Chlorine is used in waste water treatment process for disinfection purpose. Being a strong oxidant, chlorine is effective in iron and manganese removal, destruction of pathogens and elimination of ammonia. Liquid chlorine is used in most water treatment plants in the United States, due to higher expense of hypochlorite. Chlorine would react with water to form hydrochloric acid and hypochlorous acid.

$$Cl_2 + H_2O \rightarrow HCl + HOCl \qquad (1\text{-}1)$$

Above pH 8, the majority of chlorine species is in the form of OCl^-, whereas below pH 7, most of the HOCl remains unionized. However, free molecular chlorine does not typically appear in waste water treatment effluent, because of the high concentration of ammonia in treated waste water (10-15 mg/L). Chlorine reacts with ammonia in water to form monochloramine, dichloramine and trichloramine. The sum concentration of those three species are referred to as combined chlorine. The most common application of chlorination is disinfection of drinking water to destroy microorganisms that cause water borne diseases in

humans such as typhoid fever, cholera, and dysentery. Outbreaks of these diseases are caused by contaminated ground water or by inadequate or interrupted chlorination of municipal water supplies. No outbreaks of water borne diseases have been detected after proper treatment by chemical coagulation, filtration, and chlorination, which proves that current treatments are effective to prevent those diseases [5].

Safe Drinking Water Act of 1970

Chemical contamination did not become a public health concern in the United States until 1970, culminating in the passage of the Safe Drinking Water Act (SDWA) of 1974 [6]. The purpose of the safe drinking water act of 1970 is to protect public health by regulating the country's public drinking water supply. From then on, USEPA was authorized to develop enforceable standard. Originally, the issues of 22 well-known chemical including trihalomethanes (THMs) and microbial contaminants were addressed under the 1974 safe water drinking act. Later, the Safe Drinking Water Act of 1974 was amended in 1986, and a strict regulatory schedule for USEPA was established. As a result, the number of regulated contaminants increased from 22 in 1975 to 84 in 1992. In 1996, the SDWA was amended to establish a process of identifying, monitoring, and establishing maximum acceptable concentrations of new contaminants. The SDWA amendment defined contaminants of regulatory concern as compounds that have adverse human health effects and that are detected at sufficiently high concentrations to cause public concern.

Analyzing Pollutants in Waterways around Chicago

Chicagoans rely on Lake Michigan as the drinking water source, so it is vital to keep the Lake free of contaminants that pose a health risk. It is assumed there are many potentially harmful pollutants present in natural water, whose chemical and physical properties are not present in

databases and may have unknown chemical structure. Therefore, the goal of this study is to analyze the pollutants in waterways around Chicago, including Lake Michigan and the Chicago River.

Chicago Sewer System

In order to understand how polluted wastewater from sewers may contaminate Lake Michigan and the Chicago River, it is necessary to describe the Chicago sewer system. The Chicago sewer system is a combined one, which transfers both the sanitary sewage from homes, businesses and rain storm water drain to the same municipal sewer, then flow into the Metropolitan Water Reclamation District of Great Chicago (MWRD) intercepting sewers, whose diameters can be as large as 27 feet. The combined sewage is then transported from intercepting sewers to water reclamation plants (Stickney, Calumet, and North Side) for treatment. One problem arises from such design suffers overflow during heavy rain, since the amount of combined sewage and rain water may exceed the capacity of intercepting sewers. The surplus, untreated water is going to overflow into the Chicago River and the microorganisms, chemicals may pose serious effect to water environment. Another problem raised more concern about contaminating Lake Michigan, the drinking water source. It is well known that the flow of Chicago River had been reversed in order to protect the ecosystem of Lake Michigan at the beginning of twentieth century. The reason that induced the inversion of Chicago River flow was the outbreak of waterborne diseases (Cholera, typhoid) following a heavy rainstorm in 1885 [7]. That rainstorm caused the Chicago sewer system to overflow and the sewage flushed into Lake Michigan beyond the clean water intake. The accident led to the death of 12 percent of the population of approximately 700,000, and resulted in the proposed building of the Sanitary and Ship Canal (SSC) in 1889. The Sanitary

and Ship Canal, inversed the Chicago River flow toward Lake Michigan (Figure 1). Instead, the water in the canal was to flow into the Des Plaines, which combines with the Kankakee to become the Illinois River, which in turn flows into the Mississippi River.

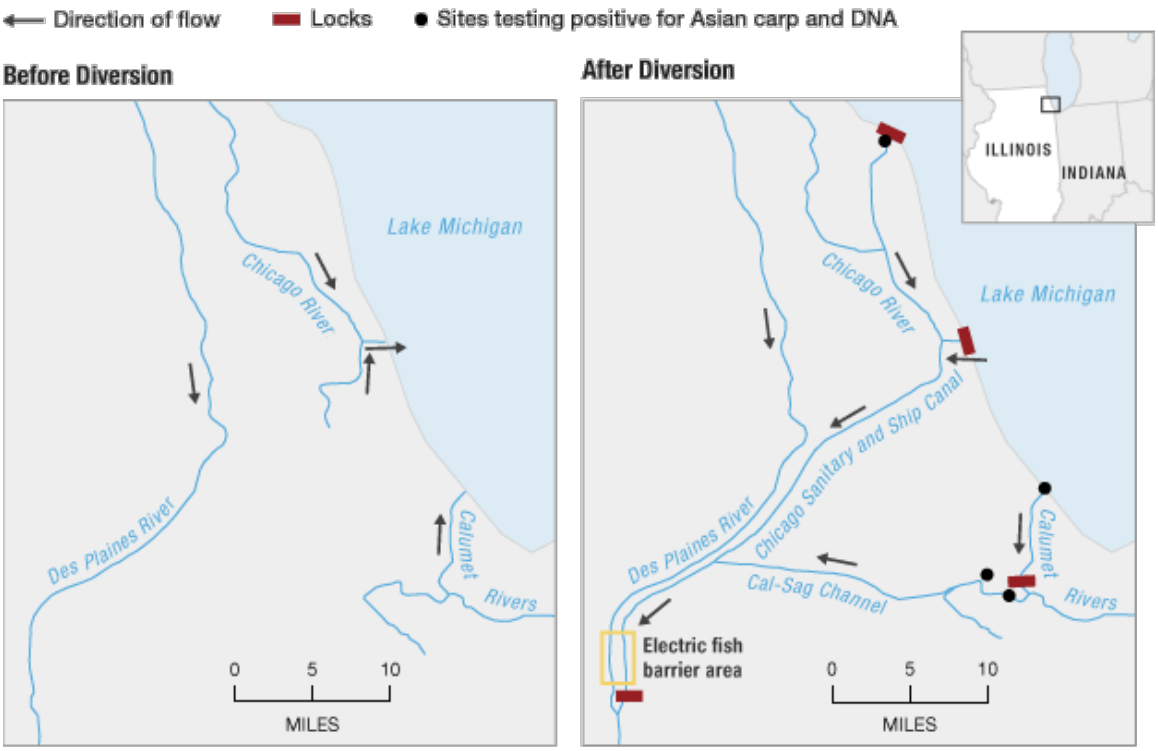


Figure 1. Indication diagram of inversion of Chicago River flow (http://www.npr.org/templates/story/story.php?storyId=130400272)

The building of Sanitary and Ship Canal was the largest public works excavation project ever undertaken at that time. The aftermath of the inversion of Chicago River flow effectively prevented the contamination of Lake Michigan from the sewage in Chicago River. However, the construction of Chicago sewer system was well before the building of waste water treatment plants (approximately one hundred years before), and the volume of modern home, business and industry waste water is much larger than that generated two hundred years ago, due to the large

population and modern life style. So the risk of overflow of Chicago sewer system still exists during heavy rainstorm in the 21st century.

Ever since the operation of deep tunnel in 1984, the deep tunnel project has effectively decreased the frequency of overflow that used to happen about twice per year on average. The temporary reservoirs will hold up to 20 billion gallons of waste water.

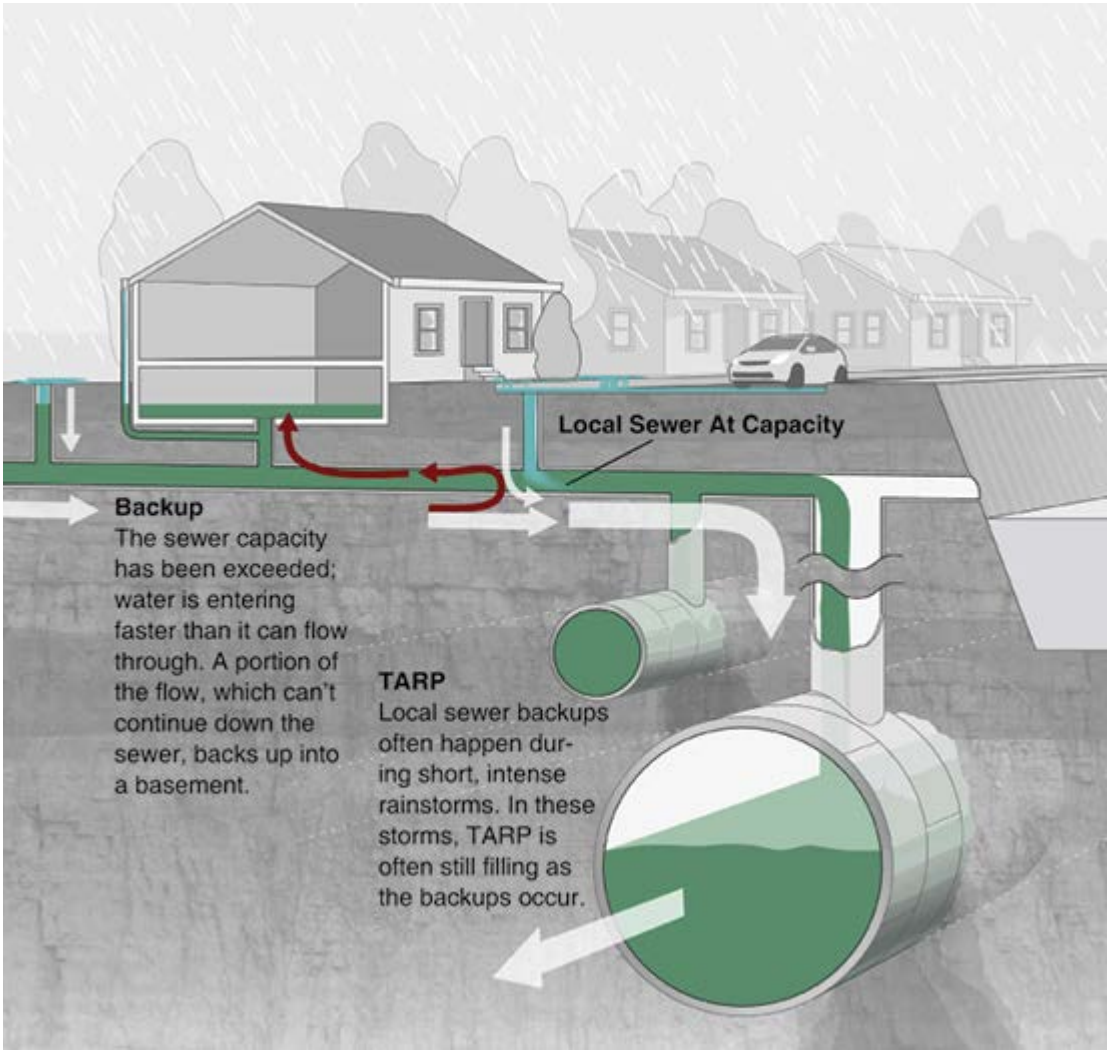


Figure 2. Diagram of TARP (Tunnel and Reservoir Project), the "Deep Tunnel" (http://www.Chicagobungalow.org)

Compound Classes of Greatest Concern

As the 20th Century progressed, an avalanche of new consumer products hit the market bringing with them a whole host of new compounds entering the water supply whose possible health effects have been a concern. With the continuous introduction of new consumer products such as shampoos, hand soaps, medicines, sun screens, the quantities of potential pollutants keep increasing. This class of chemicals are composed of pharmaceuticals (both illicit and legal) and active ingredients in personal care products (PPCPs) and receive comparatively little attention. The chemical disinfection of drinking water was a major public health improvement that was achieved at the beginning of the 20th Century. In the process of water treatment, disinfection byproducts (DBPs) are formed and raised great concern as potential pollutants.

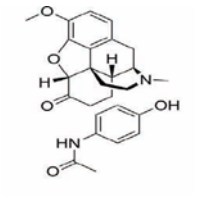
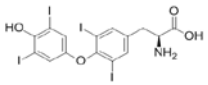
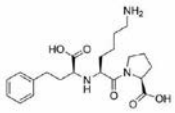
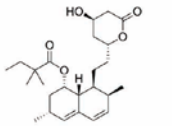
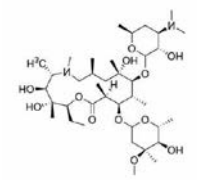
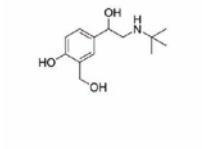
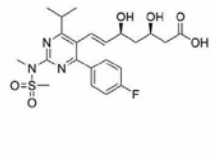
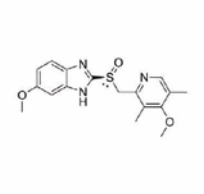
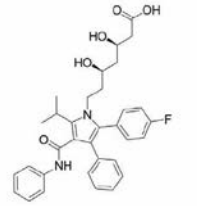
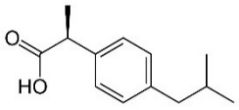
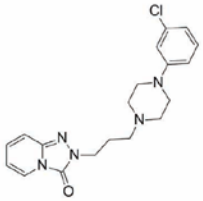
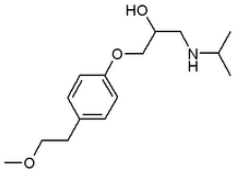
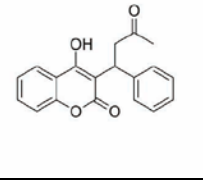
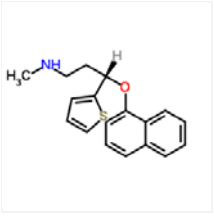
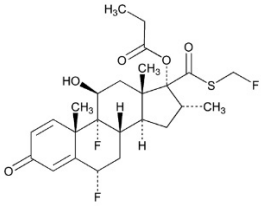
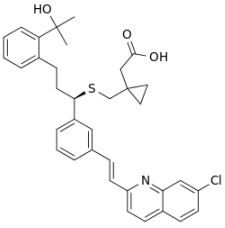
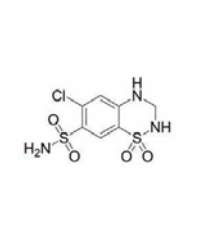
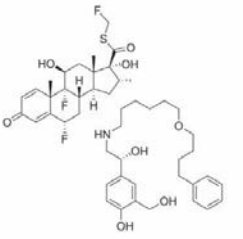
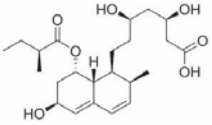
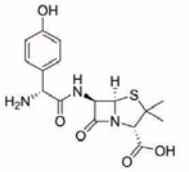
Pharmaceuticals and Personal Care Products

Pharmaceuticals. Pharmaceuticals raised people's concern due to the increasing prescription of medicines to treat different kinds of diseases. The top 20 most prescribed pharmaceuticals in 2012 are ranked in Table 1 [9] below, which include analgesics (1, 9), antiarthritic (2), antihypertensive (3, 11), anticholest (4, 8, 19), antibacterial (5, 20), stimulants (6), antiulcerants (7), antidepressant (10, 13), anticoagulant (12), corticoids (14, 18), anti-asthmatics (15), angioten-II (16), and B2-stimulants (17). When the pharmaceuticals are discharged to the water environment, it is anticipated that any of these pharmaceuticals and some of their metabolite transformation products might be identified as being present in water. Chlorine atoms are often used in pharmaceuticals because the lipophilicity and polarizability they impart to these molecules often facilitates receptor binding in the targeted tissues and pharmacological functions and because chlorine atoms block metabolism. In the forty top

pharmaceuticals those are prescribed most in 2012, nine pharmaceuticals contain at least one chlorine atoms. Due to the strong carbon-chlorine bond, these pharmaceuticals may be persistent into the water environment for long periods of time without sufficient treatment. Other pharmaceuticals, such as azithromycin, although does not contain chlorine, are being discharged into water system with molecular structure intact. This compound has been detected in waste water effluent in southwest Kentucky [10]. It can kill bacteria and other microorganisms that are essential to the food web and thus change which species dominate in the ecosystem. Furthermore, bacteria that exist in the environment could easily evolve in such a way that they are resistant to azithromycin (and other antibiotics) and thus rendering it ineffective for treating humans. This means that the widespread usage of azithromycin and other antibiotics will make us more prone to die of diseases that are treated by azithromycin/other antibiotics.

Steroids were the first class of compounds to be reported in sewage effluent. Effluent from wastewater treatment plants in Germany were found to contain drugs and drug metabolites and then in rivers and streams, with human excretion as the primary source [11]. Some pharmaceutical such as clofibric acid (blood lipid regulator) is ubiquitous and persistent in the environment. Clofibric acid (2-(4-chlorophenoxy)-2-methyl propanoic acid), a metabolite of the cholesterol-lowering pharmaceutical drug clofibrate, was the first prescription drug that was reported in a sewage effluent. Analgesics/Anti-inflammatory drugs such as diclofenac, ibuprofen, acetylsalicylic acid, and ketoprofen were found in sewage, river water, effluent. Beta-blockers such as metoprolol and propranolol were also found in surface waters at concentrations just above the limit of detection.

Table 1. Top 20 Pharmaceutical Products by US Prescription in 2012

1 Hydrocodone 	2 Levothyroxine 	3 Lisinopril 	4 Simvastatin 
5 Azithromycin 	6 Proair HFA 	7 Crestor 	8 Nexium 
9 Atorvastatin 	10 Ibuprofen 	11 Trazodone 	12 Metoprolol 
13 Warfarin 	14 Cymbalta 	15 Fluticasone propion 	16 Singulair 
17 Hydrochloro thiazide 	18 Advair Diskus 	19 Pravastatin 	20 Amoxicillin 

Personal care products. Personal care products include shampoos, skin care products, hair sprays, soaps, sun screens, and perfumes. In contrast to pharmaceuticals, scientists paid almost no attention to their environmental fates and possible environmental adverse effects. Among them, fragrances (musks) are persistent and bioaccumulative since they are slow to biodegrade. Musks have been found in the Elbe River, Germany [12] at concentrations similar to polycyclic aromatic hydrocarbons (PAHs), hexachlorobiphenyl, and p, p'-DDT.

Sunscreen Agents (UV filters) were often detected in fish from small lakes used for recreational swimming. The high lipophilicity nature leads to the bioaccumulation of sunscreen agents in fish tissue.

In a summary, some PPCPs are persistent in the environment such as blood lipid regulators and musks. Also, many pharmaceuticals those have been used to cure diseases could have unforeseen effects on nontarget organisms. The effects could be initially imperceptible and unpredictable. Thus, it may take long periods of time until the effects become profound and noticeable.

Disinfection Byproducts (DBPs)

Disinfection byproducts are formed as a result of the reaction between chlorine and organic substances naturally present in water. Six-hundred DBPs have been reported so far [13]. There are several classes of DBPs including trihalomethanes (THMs), haloacetic acids (HAAs). THMs and HAAs are the two major classes of DBPs, attributing approximately 25% of all the DBPs [14], and additional classes include haloacetonitriles, haloacetates, haloketones, haloaldehydes, halonitromethanes and haloamides etc.

THMs. Trihalomethanes (THMs) are one class of DBPs, where three of the four hydrogen atoms have been replaced by three atoms of chlorine, bromine, or iodine. Chloroform, the first DBP, was identified using GC-MS in 1974. The THMs have been studied intensively in the past thirty years, not only by analytical chemists, but also by biologists. Chloroform, bromodichloromethane, chlorodibromomethane and bromoform are all found to be carcinogenic in rodents. Administered in drinking water, bromodichloromethane produced liver tumors, and chloroform produced renal tumors in rats [15]. Since large populations obtain their water from municipalities using chlorine as the primary source of disinfection, they are exposed to low levels of bromodichloromethane constantly, increasing the risk of cancer.

HAAs. Haloacetic acids are second to trihalomethanes as the most frequently detected disinfection byproducts in surface drinking water supplies in US. Bromoacetic acid, dibromoacetic acid, chloroacetic acid, dichloroacetic acid and trichloroacetic acid are the five haloacetic acids that are regulated by the US EPA currently. Haloacetic acids (HAAs) are formed by disinfection with chlorination as well. Among these five haloacetic acids, dibromoacetic acid [16], dichloroacetic acid and trichloroacetic acid [17] have produced tumors after drinking-water exposures.

Compared with most PPCPs, the adverse effects of THMs and HAAs have been studied more thoroughly. They are also being regulated by EPA.

There are 15,000 new compounds that are added to Chemical Abstracts (CA) each year, over 89 million compounds are listed in CA and 65 million compounds are commercially available [18]. When compounds enter the water environment, they may transformed into species with unknown, unanticipated structures. The hypothesis here is that there are pollutants with

unknown structures present in Chicago metropolitan area water supply that are potentially toxic. The goal of this study is try to detect those contaminants and identify them and to propose possible remedies to remove them.

CHAPTER TWO

CURRENT STRATEGIES AND METHODS FOR ANALYZING KNOWN POLLUTANTS AND EMERGING CONTAMINANTS IN WATER

Instrumentation

The instrumentation used for this study is based on liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS), the latter is also called LC tandem mass spectrometry. There are two kinds of mass spectrometers utilized here: One is a Triple quadrupole mass spectrometer and the other is a quadrupole time-of-flight (TOF) mass spectrometer. Their principles will be explained below.

Triple Quadrupole Mass Spectrometer

In order to illustrate the function of quadruple, a single quadruple mass analyzer diagram is shown below in Figure 3. As the name implies, a single quadruple consists of four cylindrical rods that are parallel to each other. Two opposite rods carry a positive charge, while remaining two rods carry a negative charge. When a positive ion enters the space between the rods, it will be drawn towards a negative rod, and vice versa. Ions traveling inside a total electric field that is formed by a quadrupolar alternative field superposed on a constant field resulting from the application of the potentials upon the rods:

$$\Phi_0 = \pm (U - V\cos \omega t) \quad (2\text{-}1)$$

Where Φ_0 is the potential applied to the rods, ω represents the angular frequency with the unit of radians per second; $\omega = 2\pi\nu$ (ν is the frequency of the RF field), U is the DC voltage, and

V is the amplitude of the RF voltage (between 0 and peak voltage). Quadrupole mass analyzer is designed in such a way that only ions (M1) with certain mass-to-charge (m/z) could pass the rods and reach the detector. Ions (M2 and M3) with other m/z will be discharged on the rod and cannot be detected.

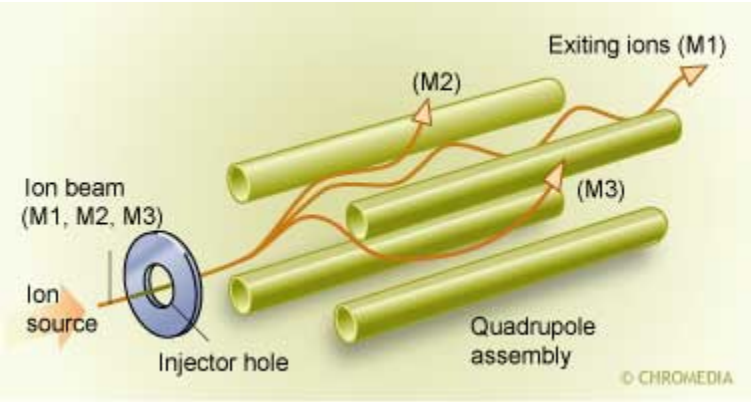


Figure 3. Diagram of ion trajectories in a single quadruple mass analyzer (http://www.chromedia.org)

A single quadrupole may be used for full-scan (molecular weight analysis) and single ion monitoring (SIM-for quantification) as well.

The triple quadrupole mass spectrometer is composed of two quadrupole mass spectrometers, with a radio frequency (RF) only quadrupole between them to act as a collision cell (q). The first quadrupole (Q1) and third quadrupole (Q3) are mass filters. A triple quadrupole mass spectrometer diagram is shown in Figure 4.

The quadrupole mass spectrometers are symbolized by upper case Q, and the RF-only quadrupole (collision cell) with a lower case q, thus a triple quadrupole mass spectrometer is written as QqQ. A collision gas, nitrogen or helium, is introduced in the central quadrupole, causing the ion to undergo collisions, fragment, and be detected by the quadrupole Q3. There are several scan modes that could be performed on a triple quadrupole mass spectrometer in

addition to the two scan modes described above. These include product ion scans, precursor ion scans, constant neutral loss scan and selected reaction monitoring (Figure 5).

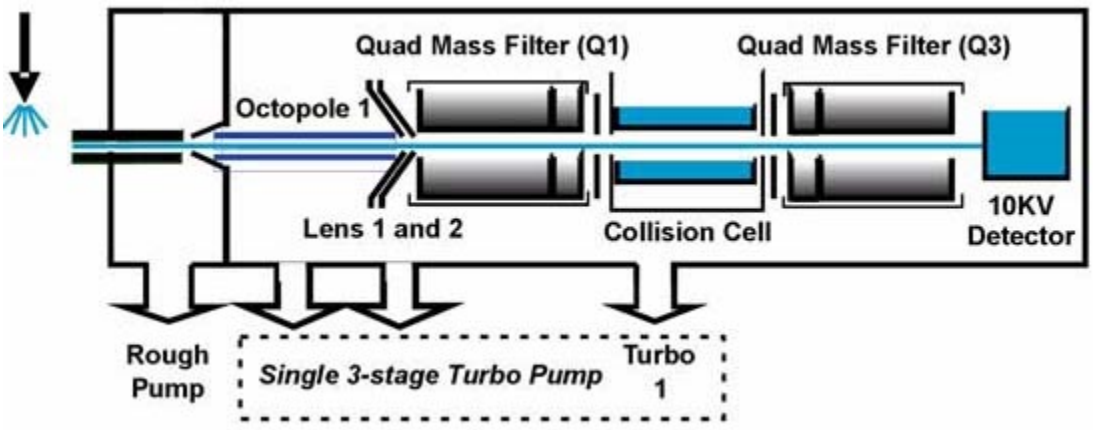


Figure 4. Diagram of a triple quadrupole instrument (http://www.kprime.net/pdf/products/6400_Data_Sheet.pdf)

The first scan mode is called product ion scan: product ion scan involves selecting a parent ion with a chosen m/z using the first mass analyzer. The precursor ion then collides with an inert gas (N_2 or Argon) where translational energy is transferred to internal modes that enable bond cleavage. These product ions are detected by the second mass analyzer.

The second scan mode is called precursor ion scan. Precursor ion scan involves detecting a specific m/z value with Q3 while scanning the first spectrometer (Q1). The ions those produce the selected ion through fragmentation thus will be detected. Only precursor ions that fragment to give the m/z value selected by Q3 are detected.

The third scan mode is called constant neutral loss scan. In this scan mode, both mass spectrometers are scanned at the same rate, but with a constant mass offset between these two. Thus, for a mass difference a, when an ion with mass x goes through the first mass spectrometer, the ion that can be detected by the second mass spectrometer has the mass of x-a. For example, a

chlorine containing compound can be detected by scanning the neutral loss of 36 mass units (HCl).

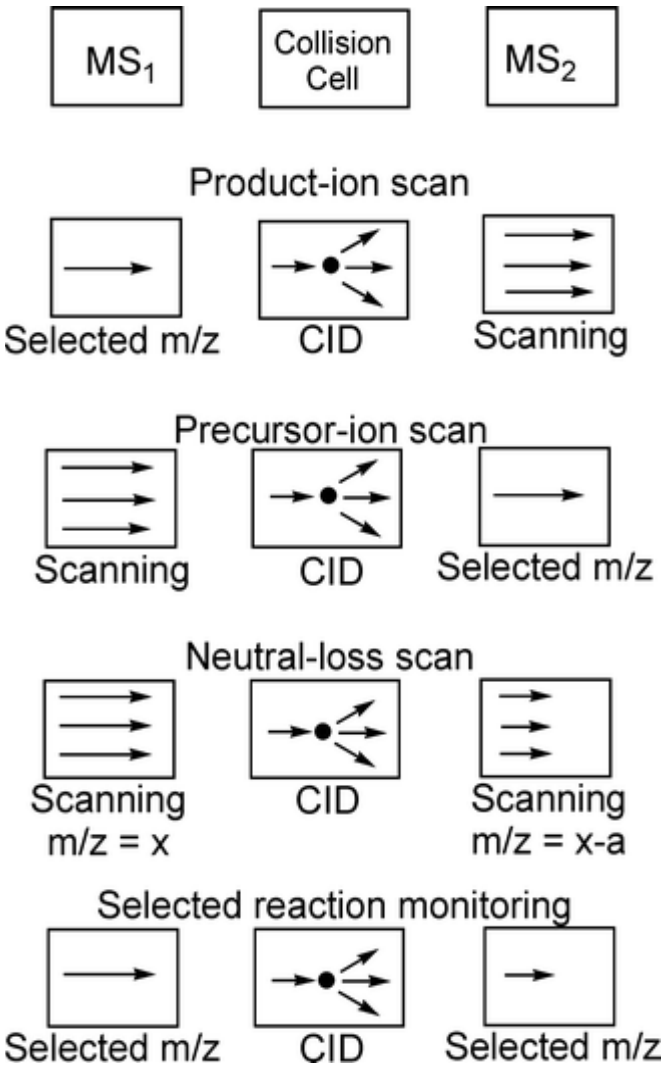


Figure 5. Different scan modes for a tandem mass spectrometer (http://pubs.rsc.org/en/content/articlehtml/2009/cs/b618553n)

The forth scan mode is called selected reaction monitoring (SRM). This method is used to perform trace analysis of targeted compounds. SRM is accomplished by selecting a particular m/z value (parent ion) with the first mass spectrometer, and then a m/z value of an ion product of a fragmentation reaction of the parent ion is selected in the second mass analyzer for detection.

Triple quadrupole mass spectrometer has the following strength: good reproducibility, relatively inexpensive, fast scanning, and it is well suited for chromatographic detection. The biggest drawback is the lack of mass resolution (limited to unit mass discrimination) associated with a mass analysis.

Time-of-Flight Mass Spectrometer

A time-of-flight (TOF) mass analyzer (Figure 6) was first described over 60 years ago [19], however, it is only recently (in the 1990s) that TOF has really taken off, as it achieves excellent resolution along with its traditional advantages such as large mass range, high duty factor (ratio of ions detected/total ions formed).

The principle of the TOF operation is straightforward; a beam of ions is generated in the source, and accelerated through an electric field into the TOF analyzer. Essentially, those ions possess the same kinetic energy. Therefore, its velocity is inversely related to its square root of mass. The ions travel along a straight line in a drift tube until they are detected. Since their masses are different, their arrival times are different too, which can be transformed to mass spectrum.

$$v = \sqrt{\frac{2KE}{m}} \tag{2-2}$$

where KE is the kinetic energy, m is the mass and v is the velocity.

The kinetic energy obtained by each ion is equal to the product of the charge of the ion and the electric field strength.

$$KE = zeV \tag{2-3}$$

where z is the charge number, e is the charge of an electron in coulombs, and V is the strength of the electric field in volts. By solving equation 2-2 and 2-3,

$$m/z = 2eVt^2/l^2 \quad (2\text{-}4)$$

where t is the flight time and l is the flight path length in meters.

However, the ions leaving the ion source of TOF have neither exactly the same starting times nor exactly the same kinetic energies. The resolution of the TOF analyzer is limited by the initial velocity spread of the ions, and the spread in starting times for ions with the same m/z value is often larger than the difference in time of flight of ions with different m/z values. Therefore, the difference of initial kinetic energy is going to affect the resolution significantly. A way to improve mass resolution is to use a reflectron.

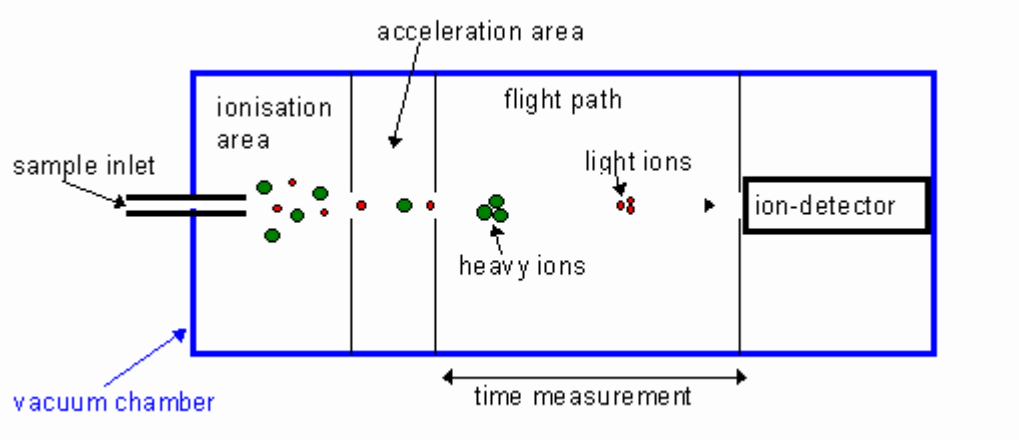


Figure 6. Time of flight mass spectrometer indication diagram (http://alevelnotes.com/Mass-Spectrometry/124)

Reflector time-of-flight analyzer. An electrostatic reflector, also called a reflectron is used to improve mass resolution. A reflectron is an optical device in which ions in TOF interact with ion mirror and their flights are reversed. A simple reflectron consists of a retarding electric field behind the free drift region. The ions penetrate the reflection until their velocity reach zero and are then sent back from the reflectron in opposite direction. Ions with greater kinetic energies travel further into the reflectron than ions with smaller kinetic energies. The ions that travel

further will take longer time to return to the detector. Better separation/resolution for ions having different m/z values is obtained but the initial thermal velocity spread of isomass ions is maintained. Ion trajectories in a reflectron-TOF are shown below in Figure 7.

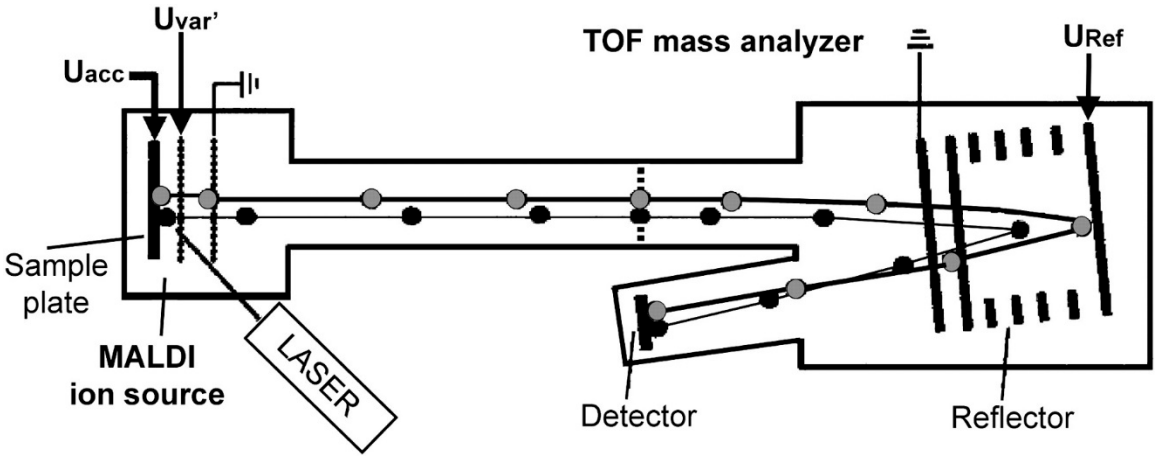


Figure 7. Indication diagram of reflectron in TOF mass analyzer (http://imgkid.com/mass-spectrometer-schematic.shtml)

Quadrupole Time-of-Flight (QTOF) Mass Spectrometer

Quadrupole Time-of-Flight (QTOF) is a hybrid instrument combines the simplicity of the quadrupole and the high performance of the TOF. The high sensitivity and high mass accuracy can be achieved in both MS and tandem (MS/MS) modes. The instrument can be looked as a triple quadrupole except that the last quadrupole is replaced with a TOF. In MS mode, the quadrupole acts only as the ion guide, and the TOF does all of the analysis. In MS/MS mode, the Q1 in Figure 8 is used to transmit and to select precursor ions of interest. They undergo fragmentation in Q2 by collision with neutral gas (nitrogen or argon). The fragments are then being analyzed by TOF. The resolution of QTOF ($m/\Delta m$) can be as high as 50,000, enabling mass measurement accuracy of 5 to 10 ppm. High mass resolution is essential for accurate mass analysis.

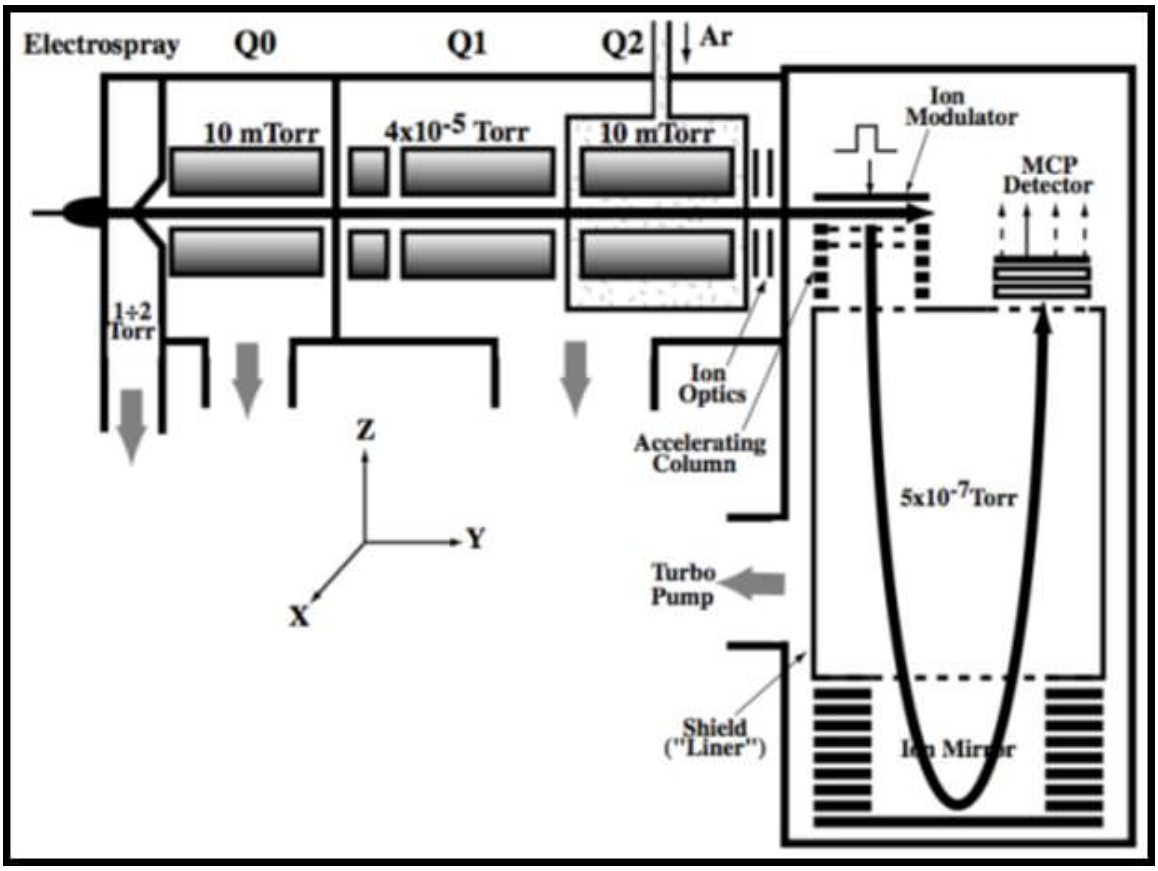


Figure 8. Indication diagram of QTOF (http://msr.dom.wustl.edu/wp-content/uploads/2014/07/figure-19.jpg)

Here accurate mass analysis of product ions ($\Delta m = 0.1$ mDa) is possible, allowing for a more unambiguous identification of product ion empirical formulas.

Ionization Methods-Electrospray Ionization (ESI)

Electrospray ionization (ESI) is well suited as an ionization method for LC/MS applications, because ions are formed from the eluting liquid phase. Ions are produced when a voltage of 3-6 kV (shown in Figure 9), is applied to the end of the capillary where the mobile phase is aspirated into the high vacuum. The mobile phase produces charged droplets as it exits the needle. At the tip of the needle, a “Taylor cone” is formed by the interaction of surface tension and electrostatic Coulomb force. When the analyte solution is exposed to an electric

field, the shape of the liquid starts to deform from the shape caused by surface tension alone. As the voltage increases, the effect of electrostatic Coulomb force is becoming prominent, until a certain voltage is applied and an equilibrium between surface tension derived force and electrostatic force is reached, in other word, the “Taylor cone” is formed with an open angle of 49.3° [20]. Spray droplets formed during the ESI process are then repelled from the needle towards the source, as the droplets travel through the space between the needle tip and the cone, solvent is evaporated. As the solvent evaporation happens, the droplet shrinks until it reaches the point that the surface tension could no long sustain the charge (the Rayleigh limit), which results a “Coulombic explosion” and the droplet is broken apart. Solvent continues to evaporate from the droplet until only the ions remain.

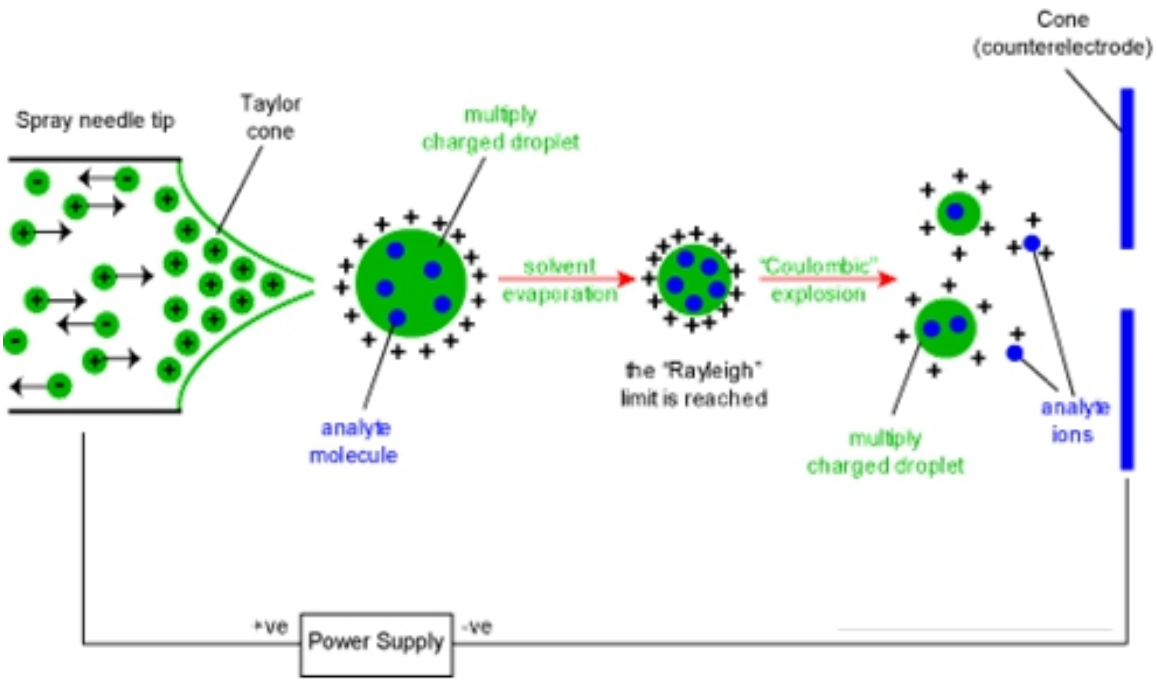


Figure 9. Scheme of electrospray formation mechanism
(http://www.bris.ac.uk/nerclsmsf/techniques/hplcms.html)

ESI is a soft ionization method, since very little internal energy is retained by the analyte upon ionization. Therefore, ESI could be utilized to analyze biomolecules such as peptides, proteins etc.

Current Strategies for Analyzing Known Pollutants and Emerging Contaminants

Quantification of Known Pollutants

Targeted analyses are carried out by tandem mass spectrometry based on detection of two fragmentation reactions, one for quantification and the second transition for verification. Specifically, multiple reaction monitoring (MRM) is utilized here, and two transitions are monitored. The quantification of known pollutants requires the usage of isotope-labeled standards and the method is called “isotope dilution”. Isotope dilution analysis is a method of determining the quantity of target analyte. Here a known amount of the target compound labeled with several deuteriums or carbon-13 atoms is added to the sample being analyzed. The isotope-labeled standard and the analyte are assumed to ionize and be detected with the same efficiency because they have isotopic chemical structures. This means that the peak area ratio of the labeled standard and analyte directly reflects the ratio of their relative abundances.

An isotope dilution method has been developed for the trace analysis of 15 pharmaceuticals, four metabolites of pharmaceuticals, three potential endocrine disruptors, and one personal care product [21] in waste water influent and effluent obtained from water pollution control facility in Las Vegas, NV, and finished drinking water from River Mountains Water Treatment Facility. The isotope dilution method used Atorvastatin-d_5, Bisphenol A-d_{16}, Diclofenac-d_4, and Triclosan-d_3 etc as the isotope labeled standards, and most of compounds in the above paper were quantified in waste water influent above 1000 ng/L.

Although the isotope dilution has the advantages of ideal internal standard method, correction for signal drift, correction for matrix effects, compensated losses of substances, excellent precision and accuracy, it does have some disadvantage, such as limited availability of isotope labeled standards, especially for the detection of emerging contaminants (ECs), since the identity of the pollutants may be unknown.

Analysis of "Known Unknowns" to Detect Emerging Contaminants (ECs)

The method for analysis of "Known Unknowns" to detect emerging contaminants (ECs) is also called the non-target method, differentiated from the above isotope dilution method for quantification of known pollutants. Non-target screening methods such as these are referred to as the analysis of "known unknowns" [22]. The empirical formulas derived from the accurate mass analysis are searched in different databases to verify the presence of emerging contaminants. Emerging contaminants may be broadly defined as compounds that have been detected in the environment for which no regular environmental monitoring is being carried out and no toxicological data exists. ECs are either known or suspected of exerting adverse human health effects and/or ecological effects. Analysis of known unknowns is carried out not to quantify them, but to judge the extent of "spread" of ECs through the environment. Several prominent examples are described below.

Polybrominated diphenylethers (PBDEs), commonly found in flame retardants, have been shown to affect endocrine systems (regulate the body's hormones) [23].

Triclosan and triclocarban, antibacterial chemicals commonly found in PPCPs ranging from liquid hand soaps to toothpastes to clean products, are suspected endocrine disruptors [24] and are toxic to fish.

Bisphenol A, commonly found in plastics ranging from food storage containers, metal can liners to water bottles, has been found to be endocrine-disrupting [25].

Time-of-flight methods. The vast majority of ‘Known unknown” analyses are carried out using a Q-TOF mass analyzer [26]. Due to the high complexity of some environmental samples such as waste water and sludge samples, high resolving power techniques are needed to provide additional molecular weight information. Complex matrix environmental sample analysis can benefit from this enhanced resolving power, by separating the isobaric interferences from unknown of interest. Furthermore, the Q-TOF mass spectrometry is one of the high resolution mass spectrometry (HRMS), and its main feature is its ability to obtain accurate masses within 5ppm of the theoretical mass. Except for the capability of the measurement of accurate mass, Q-TOF mass spectrometry also has the advantage of collecting data across a wide mass range without losing sensitivity, so that a full spectral sensitivity is achieved.

In environmental analysis, the number of emerging contaminants present in a sample may be enormous. As a result, in the past five years, the applications of Q-TOF detection have been mainly confined in qualitative analysis [27] and in the form of non-target screening of emerging contaminants. The intrinsic characteristics of LC-QTOF-MS enable screening of a vast number of contaminants with high sensitivity within one injection.

Other mass analyzers. Fourier Transform Ion Cyclotron Resonance (FTICR) is a high resolution mass analyzer in use to solve environmental problems. The principle (Figure 10) is to apply a magnetic field B, which is oriented along the Z axis, and is intense enough to trap the ion with certain velocity on a circular trajectory in the trap. The ions rotate in the XY plane

perpendicular to the Z axis. An ion trapped in a magnetic field B is described below in equation 2-5.

$$mv = qBr \tag{2-5}$$

In which m, q, and v are mass, charge, and velocity, and r is the radius of the ion making circular movement in XY plane. The angular velocity, ω (in radian/sec), can be defined in equation 2-6.

$$\omega = \upsilon / r \tag{2-6}$$

From equation 2-5 and 2-6, relationship of ω to mass charge ratio can be deduced.

$$\omega = {}^{qB}/_{m} \tag{2-7}$$

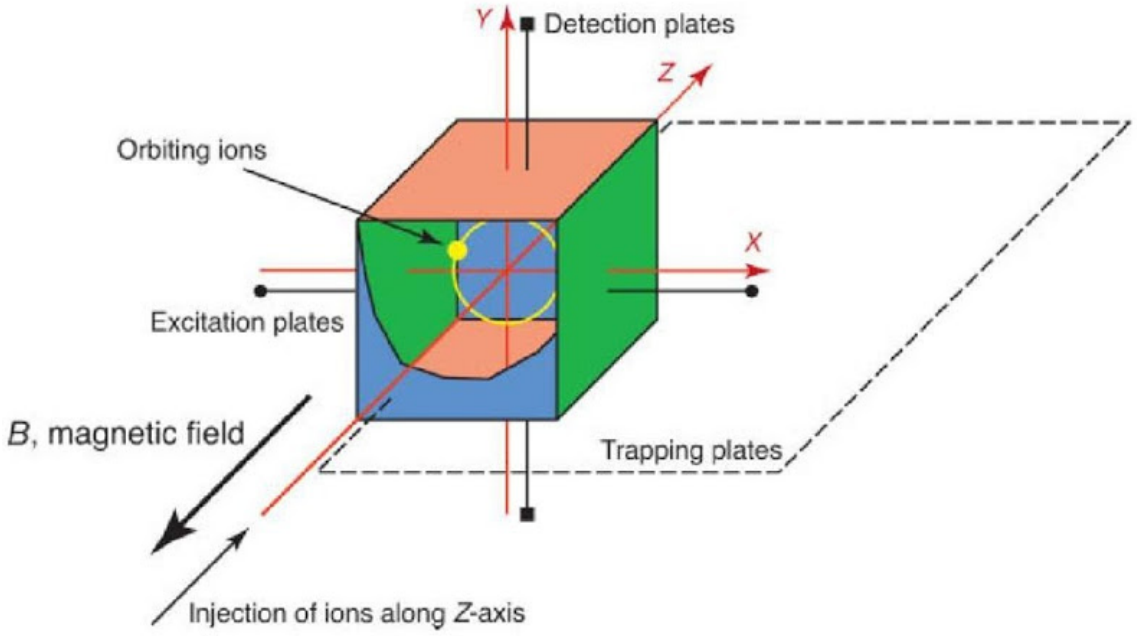


Figure 10. Scheme of ion cyclotron resonance (http://what-when-how.com/proteomics/ft-icr-proteomics/)

Since f, the frequency, equals angular velocity divided by 2π, therefore, the frequency of the ion is dependent on q/m (m/z) only. Excitation of the ions is achieved by applying an electric field oscillating at the cyclotron frequency of ions of a particular m/z value. Thus, ion cyclotron resonance (ICR) occurs when the ions are irradiated with an electromagnetic wave (usage of a radio frequency potential on two excitation plates) that has the same frequency as the ion's cyclotron frequency. Upon absorbing the wave, the ion's trajectory radius is increased and is excited, eventually, the ions can be excited to detectable ICR orbital radius. Other excitation modes include frequency-sweep, stored waveform inverse Fourier transform (SWIFT) etc [28].

When the ions are excited to a large enough orbital radius so that the ions will pass close to the detection plates, an image current will be induced in the plate each time an ion passes by. But statistically, when one ion passes by one of the detection plates, there will be another ion that passes by the opposite plate, therefore, the resulting image current will be zero.

Detection of FTICR involves simultaneously exciting all of the ions present in the cyclotron by a rapid scan of a large frequency range within a time span of about 1μs. The detecting electrodes pick up the signals generated by all the ion bundles that circle in the excited orbit, and each bundle will induce a signal each time it passes one of the electrodes and a complicated time dependent signal is obtained. By Fourier transformation of the time spectrum, a frequency spectrum is obtained, which in turn will be converted to a mass spectrum. All frequencies corresponding to the exited ions will be recorded and transformed.

FTICR has the highest demonstrated mass resolution of any mass analyzer [28-30], with m/Δm reaching 100,000,000, depending on magnetic field and other factors such as acquisition time. Marshall et al [31] utilized FTICR to study the pollutants following the BP oil spill disaster

in July, 2010. The Deepwater Horizon disaster was estimated to dump 4.9 to 5.8 million barrels of crude oil from Macondo well into Gulf of Mexico over 87 days. Their method involves measuring the chemicals present in crude petroleum, and then compared with those present in the tar ball obtained on Louisiana beach as a result of the BP oil spill. With the resolving power ($m/\Delta m$) of 1,000,000 at m/z 499, FTICR was utilized to characterize acidic (i.e., carboxylic acids, alcohols, pyrrolic nitrogen), basic (i.e., pyridinic nitrogen), and nonpolar (aromatic hydrocarbons, furans) in Macondo well petroleum at the molecular level. As a result, the elemental compositions of those compounds were obtained, since the high resolving power of FTICR combined with mass defect (the difference between the exact mass and the next nearest integer mass) sorting and isotope fine structure is sufficient to assign a unique elemental composition for each molecule. The authors have found that compounds in a tar ball obtained in Louisiana beach contained more oxygen and fewer double bonds compared with those in crude petroleum. Their work began the molecular monitoring of the largest oil spill in US history. FTICR has also been utilized for the study on transformation of natural organic matter in source water during chlorination and chlorination products [32]. In their study, 659 single-chlorine containing products and 348 two-chlorine containing products were detected in the chlorinated samples taken from three different reservoirs in China. However, only 7 molecular formulas can be found in Richardson's list of 217 chlorine containing DBPs. So FTICR is a powerful tool for the detection of chlorine-containing compounds in a complex mixture.

Another mass analyzer in use for the analysis of emerging contaminants is the Orbitrap. The Orbitrap is shown below in Figure 11. The Orbitrap consists of an outer barrel-like electrode and a coaxial inner spindle-like electrode that traps ions in an orbital motion around the spindle.

Orbitrap uses a logarithmic electrostatic field between its inner and outer electrodes, as well as a quadrupolar field between its end caps. Ions move in stable trajectories both around the central electrode (the spindle like) and in harmonic oscillation along the Z direction of the central electrode, resulting in a spiral. Typical resolving power for the commercial instrument is up to about 150,000 [33] with mass accuracy comparable to a FTICR. Orbitrap and FTICR detection is carried out not by collisions with a multichannel plate as with a TOF. Detection of ions is accomplished when ions in a circular orbit induce an alternating current in a receiver plate (FTICR) or a hyperbolic electrode. The frequency of the induced current is directly related to the inversion of the square root of m/z value of the ion.

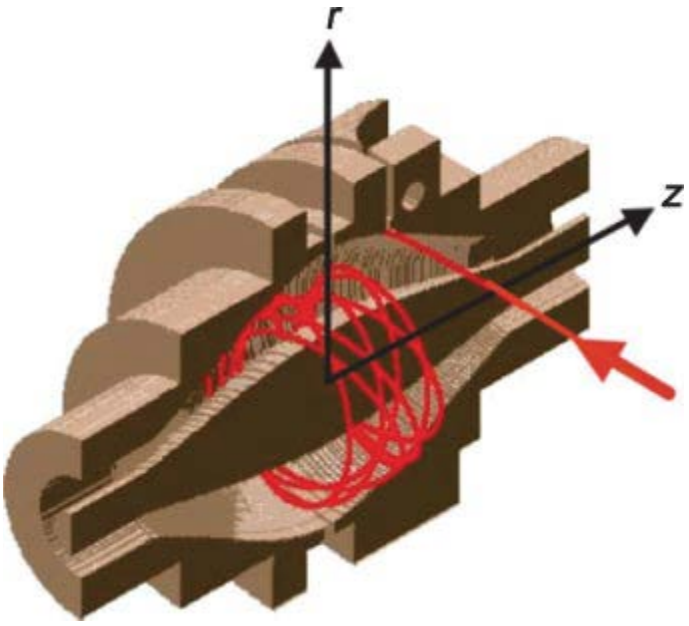


Figure 11. . Schematic of an orbitrap analyzer
(http://www.chem.umass.edu/~vachet/Course/Orbitrap_review.pdf)

Orbitraps are another prominent example of high resolution mass analyzers [34]. Orbitraps are full-scan only mass analyzers, and must be used as a hybrid instrument. The method involves full-scan screening (using orbitrap) of water samples in search of “unknown”,

followed by MS^n experiments (using linear ion trap) to elucidate the structure of the unknowns. The resolution of orbitrap in this study is as high as 100,000 and the accurate mass is recorded. By this method, clofibric acid ($C_{10}H_{11}ClO_3$) and mecoprop (methyl chlorophenoxypropionic acid, an herbicide) was identified.

Both orbitraps and FTICR instruments are capable of superior mass resolution compared to the Time-of-Flight mass analyzers. However, this superior resolution often requires longer detection times (hundreds of microseconds to a millisecond) relative to a TOF which compromises the ability of these two trapped ion techniques to provide good chromatographic peak resolution in an LC/MS analysis. Also, the high cost of FTICR instrument limits the usage of it.

Types of databases. The types of databases used in non-target analysis for ECs include Chemical Abstract Registry Service, Scifinder Scholar, ChemSpider etc. Some researchers have utilized online database searching techniques coupled with TOF method (High Resolution Mass Spectrometry) to successfully identify pollutants.

One online database that is utilized for elemental composition searching is ChemSpider. Upon obtaining accurate mass, an elemental composition is then searched against ChemSpider and the numbers of references associated with each elemental composition are listed by descending order [22]. The result would bring the most common elemental composition to the top of the list.

Another online database has been used is Chemical Abstracts Service (CAS), CAS is a particularly comprehensive source containing over 54 million entries in 2011. The database can be queried by a variety of inputs including molecular formula, average molecular weight,

structures, etc. The authors [35] queried the CAS registry by either molecular formula or average molecular weight (MW) obtained from accurate mass spectrometry data. The returned candidate list is then refined by listing number of associated references in descending order. Candidate structures are further substantiated with additional information such as product ion spectra, electron impact fragmentation patterns, nuclear magnetic resonance (NMR) data, retention times, UV-Vis spectra etc. The molecular formula is the better searching parameter for identifying a "known unknown" compared to molecular weight, however, in many actual cases, the molecular formula could not be determined and the molecular weight had to be used to search the database first. This approach has been proved to be useful to identify a variety of compounds, including polymer additives, extracts from natural products. The above approach does have its limitation, since this approach can only use the average molecular weight instead of monoisotopic mass to search.

When a number of isomers are possible for one empirical formula, the spectroscopic characteristics of the unknown must be compared to a set of isomeric standard compounds to complete an unambiguous identification. This comparison should include chromatographic retention times and product ion spectra at a minimum.

Our Research

We are postulating that many potentially harmful water pollutants may be present in natural water (lakes, rivers, etc.) whose identities are unknown (empirical formula not in a database). We wish to identify the most persistent of these unknown pollutants so that fundamental questions regarding their potential toxicity may be addressed. We are using full scan tandem mass spectrometry to detect unknown pollutants based on structural features that suggest their

potential toxicity.

The key feature of this strategy is the use of precursor ion scan to detect the chloride ion at m/z 35 over a narrow, consecutive mass range (m/z 200 to 205, m/z 205 to 210, etc) with sequential injections of sample. Such a strategy allows the determination of the molecular weights and retention times of all (and only) chloride ion (m/z 35) containing compounds with sensitivity approaching most multiple reaction monitoring experiments carried out with a triple quadrupole mass spectrometer.

After the molecular masses and retention of chlorinated compounds are established, high resolution mass spectrometry analysis (HRMS) is conducted utilizing a Quadrupole Time-of-Flight (QTOF) mass spectrometer. Once the accurate masses are obtained, they are used to calculate the elemental composition and possible empirical formulas are proposed. The empirical formulas are then searched against online databases for possible molecular structures. Many of the chlorinated species we have detected have not provided empirical formulas that listed in any database. Nevertheless, spectroscopic studies may be carried with synthetic standards to determine the identity of the unknown.

There are two reasons that this study is focusing on chlorine containing compounds. Chlorinated organic compounds, which include industrial chemicals (PCBs and polyvinyl chloride), pesticides (DDT and its derivatives), and by-products of manufacturing (dioxins), are global pollutants. In 1993, the American Public Health Association stated that “virtually all chlorinated organic compounds that have been studied exhibit at least one of a wide range of serious toxic effects such as endocrine dysfunction, developmental impairment, birth defects, reproductive dysfunction and infertility, immunosuppression, and cancer, often at extremely low

doses and that many chlorinated organic compounds, such as methylene chloride and trichloroethylene, are recognized as significant workplace hazards"[58]. The risks of these types of chlorinated compounds may be intensified because many are known to accumulate in the environment due to the stability of the carbon-chlorine bond. The EPA still monitors many of these compounds despite the fact that their manufacture was halted over 40 years ago. The sources of chlorine containing compounds that are of environmental concern now include PPCPs (pharmaceuticals and personal care products) and DBPs (disinfection byproducts).

Ions at m/z 35 and m/z 37 are specific for chlorine, so molecules containing chlorine are often easily recognizable. The relative abundances in nature of chlorine-35 and chlorine-37 are 75.77% and 24.23% respectively, so the relative intensity of the two peaks appearing in mass spectrometry is about 3:1. If the analyte is concentrated enough, high resolution mass spectrometry could show the isotopic pattern and could be used to determine the number of chlorine atoms in the unknown compound.

In summary, this study involves using a precursor ion scan to detect chlorine containing compounds, scanning a narrow, consecutive mass ranges (five Daltons) with each injection. Accurate masses are obtained by conducting high resolution mass spectrometry utilizing QTOF and then searched against online databases for possible empirical formula assignment and molecular structure. Product ion spectra are used to elucidate molecular structure. Ultimately, the spectroscopy properties (product ion spectrum and retention time) of unknown are compared with those of standard compounds.

CHAPTER THREE

EXPERIMENTAL

Overview

Water samples were collected from different locations near Chicago metropolitan areas, filtered, and unknown chlorinated pollutants isolated by solid phase extraction. The molecular masses and retention times of chlorinated analytes in the extracts were determined by precursor ion scanning for chlorine 35. Accurate mass data was acquired by high resolution mass spectrometry to calculate the empirical formulas of these molecules. Product ion spectra of these molecules were collected to get structural information about these molecules. All liquid chromatographic separations were carried out with a water/methanol gradient.

Sampling

Water samples were collected from the Chicago River and Lake Michigan (Figure 12) in 0.5-1L quantities along 100-200 ft of coastline (or pier) and combining them to obtain a total volume of 5-10 liters of sample from one site. Waste water effluent samples (20L) were collected from a tap at the Stickney waste water treatment plant in Stickney, IL USA. The sampling locations and dates are listed below in Table 2. Sampling sites were selected based in part on the population density around the immediate area and ease of access to the shoreline. The dates of sampling and the sites are summarized in Table 2.

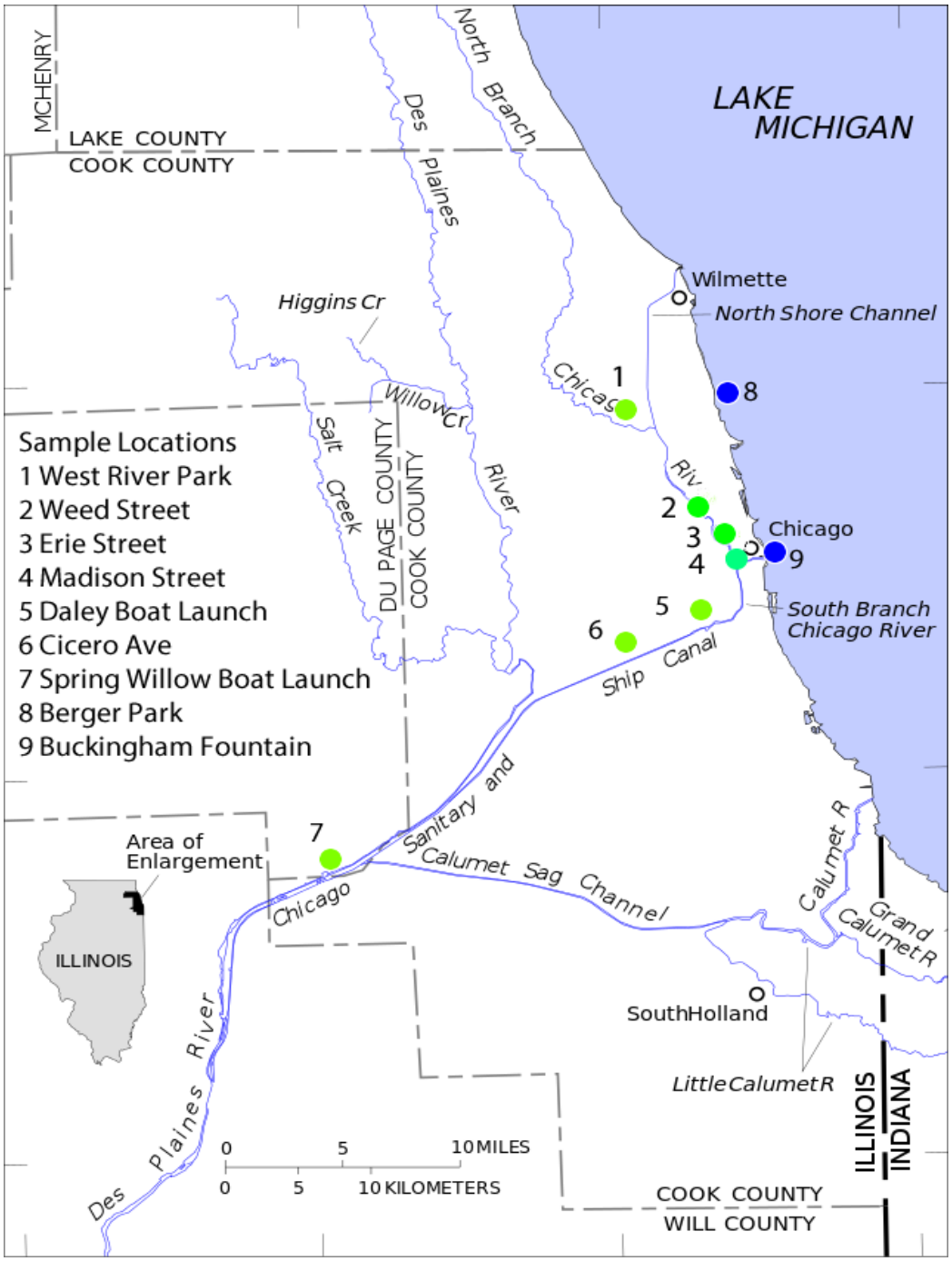


Figure 12. Sample sites along Chicago River and Lake Michigan

Table 2. Dates and Sampling Sites in and around the Chicago Area*

Sampling Sites	Date	Sampling Sites	Date
Berger Park Lake Michigan Shoreline	6/10/2013	Madison boat launch	5/09/2013 5/30/2013 (2)
Buckingham Fountain Lake Michigan Shoreline	5/09/2013 6/11/2013 (2)	West River Park	6/27/2013
Weed Street	6/27/2013	Willow Springs	7/11/2013
Daley Park	7/11/2013 7/09/2014 9/10/2014 9/11/2014 7/25/2015	Erie Street	8/14/2013 7/03/2014 7/23/2015
		Cicero Ave	8/21/2013 7/11/2014 7/24/2015
Willow Springs	7/11/2013		

* (2) means two 5-10 L samples were acquired within 15 minutes of each other from the same site.

The sampling sites were selected with the help of Mr. David Treering, a geographic information specialist in Loyola University's Institute of Environmental Studies.

In 2015, three samples were acquired from different sites on different days to do comparative product ion studies of an ion at m/z 241 suggested to be a dichlorosulfonic acid to establish whether or not compounds isolated from different locations and times had the same structure. These three samples include Erie Street (July 23rd, 2015), Cicero Ave (July 24th, 2015), and Daley Park (July 25th, 2015).

Water Extraction

Water samples were transported to the lab and were extracted on the same day. Before extraction, water samples were filtered using a Whatman 11 µm fiber glass filter paper (Whatman, MA). Oasis HLB glass cartridges from Waters (Milford, MA) (5cc/200mg) were used for extraction. Solid Phase Extraction method is conducted by the following steps [36]:

Step 1: Condition the HLB cartridge with 6 ml of MeOH with 0.25% formic acid and 12 ml of Milli-Q Water with 0.25% formic acid.

Step 2: Filter 500 ml of water sample (Chicago River or Lake Michigan or Stickney Waste Water Treatment Plant Effluent) or 100 ml of Influent.

Step 3: Adjust the PH of sample to 2 by adding concentrated formic acid (2.5 ml in every 1000 ml water).

Step 4: Connect aspirator and pass the water sample through the aspirator. Cartridges were stored at -80 ºC freezer until 1-2 days before analysis.

Step 5: Elute the cartridge with 6 ml of 0.25% formic acid in methanol.

Step 6: Dry the sample under nitrogen.

Step 7: Reconstitute the sample with 200µl of 20% methanol with 0.25% formic acid in an auto sampler vial.

Liquid Chromatography

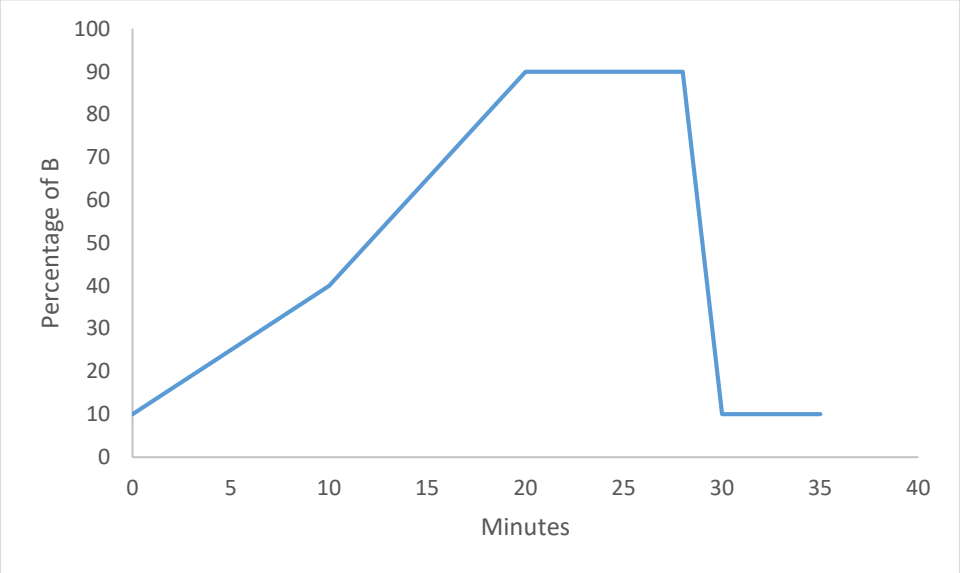


Figure 13. Gradient used for precursor ion analyses of chlorinated compounds

Separations were carried out with an Agilent 1290 Infinity UPLC system. This is interfaced to an Agilent 6460 Triple quadrupole mass spectrometer (Santa Clara, CA, USA).

Solvent A is 0.25% formic acid in Milli-Q Water and solvent B is 0.25% formic acid in methanol. The gradient starts from 10% B, reaches 40% B within ten minutes. The percentage of solvent B continues to increase for another ten minutes and is held at 90% B for eight minutes. The percentage of B decreases from 90% to 10% within two minutes and then the mobile phase re-equilibrates for five minutes (Figure 13). The flow rate for this gradient is 0.2ml/min.

A XTerra® MS C18, 5 µm (particle size) column (2.1mm × 10mm) were used for these separations (Waters Corp., Milford, MA).

Instrumentation

Precursor ion and product ion analyses were carried out with an Agilent 6460 triple quadrupole mass spectrometer (Santa Clara, CA, USA). For precursor ion analyses, consecutive, narrow mass ranges of five daltons (for example, m/z 200 to m/z 205) were scanned for precursor ions of chloride ions (m/z 35 or m/z 37) with each injection.

Eighty individual injections were made to cover a four-hundred dalton mass range (m/z 200 to m/z 600). The injection volume is 20 µl.

The configuration of the electrospray mass spectrometer is shown in Figure 14.

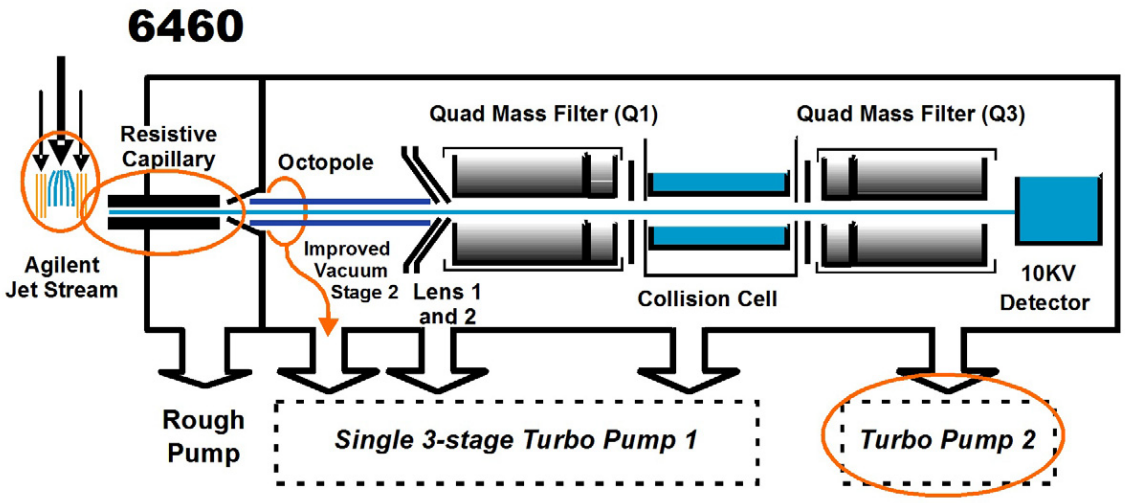


Figure 14. Configuration of Agilent 6460 triple quadrupole mass spectrometer (https://www.agilent.com/cs/library/usermanuals/Public/G3335-90135_QQQ_Concepts.pdf)

A 0.7 dalton mass window was selected for precursor ion selection for product and precursor ion analyses. The ion source tuning parameters are summarized in Table 3.

Table 3. Optimized Tuning Parameters for Precursor Ion Scan

Nebulizer gas temperature	250 °C
Shealth gas temperature	300 °C
Drying gas flow	8 L/min
Shealth gas flow	9 L/min
Nebulizer pressure	50 psi
Capillary voltage	-4500 V
Fragmentor voltage	139 V
Scan time	500 ms

The Agilent 6460 triple quadrupole is limited to unit mass resolution, therefore accurate mass measurements are required to get an unambiguous empirical formula. A Waters Synapt Quadrupole Time-of-Flight (Q-TOF) mass spectrometer interfaced to a Waters 2690 HPLC at the University of Illinois-Chicago's Research Resource Center (RRC) was used for accurate mass determinations. Sucralose (early eluting, retention time 10.5 minutes) and Triclosan (late eluting, retention time of 23.5 minutes) are used as retention time markers to compare elution

profiles of different ions analyzed with the triple quadrupole and Q-TOF MS. Both compounds are present in most wastewater effluent and natural water samples analyzed in this study. The resolution (m/Δm) for this Q-TOF is consistently 12,000 in V-mode (ion trajectory like letter V). The mass range monitored is m/z 100 to m/z 1000. The pentapeptide leucine enkephalin (Tyr-Gly-Gly-Phe-Leu) was used as a lock mass. A "lock mass" is an ion having a known m/z value (554.26151 for $(M-H)^-$ ion) that is infused with the sample, but analyzed in alternating scans. A lock mass allows for real time calibration corrections by correcting shifts in measured m/z values caused by instrument drift. The lock mass solution was infused in a solution of methanol (50 µg/mL concentration) in 1 mL Hamilton (Reno, NV, USA) syringe. The tuning parameters for the electrospray ionization source in the Waters Q-TOF are summarized in Table 4. The same column and gradient used for precursor ion analyses described above were used for accurate mass analyses.

Table 4. Parameters for Q-TOF Accurate Mass Analysis

Source Temperature	120°C
Desolvation Temperature	120°C
Desolvation Gas Flow rate	350 L/hour
Sampling Cone Voltage	77 V
Extraction Cone Voltage	102 V
Capillary Voltage	-3.4 kV
Syringe Flow Rate	10-15 µL/minute

Reagents and Materials

Milli-Q water was obtained from Barnstead E-pure milli-Q water generating system. LC grade methanol is used as the main solvent for HPLC. Formic acid (95-98%) is used as additive to the solvent. Oasis HLB (hydrophilic-lipophilic) 5 cc 200 mg cartridges were purchased from Waters (Waters Corporation, Milford, MA) for solid phase extraction of analytes from filtered water samples.

Most of the standard compounds that are used for product ion study were synthesized by Marlon Lutz in the laboratory of Dr. Daniel Becker at Loyola University Chicago. The structures are presented/discussed in Chapter 5. One standard, sodium 3,5-dichloro-2-hydroxybenzene sulfonate, was purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification.

Product Ion Studies of Dichlorophenol Sulfonic Acids

Product ion analyses have two important purposes in this study. First, by applying collision energy, the precursor ion was fragmented and the resulting product ion spectrum reveals information of the molecular structure. Second, by comparing the product ion spectrum of unknown to that of standard the identity of unknown could be confirmed. Additionally, product ion spectra may be used to provide more verification that compounds (having similar m/z values and retention times), isolated from different geographical sites have the same structure. A shorter gradient was used for the purpose of comparing product ion spectra of different dichlorophenol sulfonic acid standard compounds for similarity index studies [42-46] described in chapter 6.

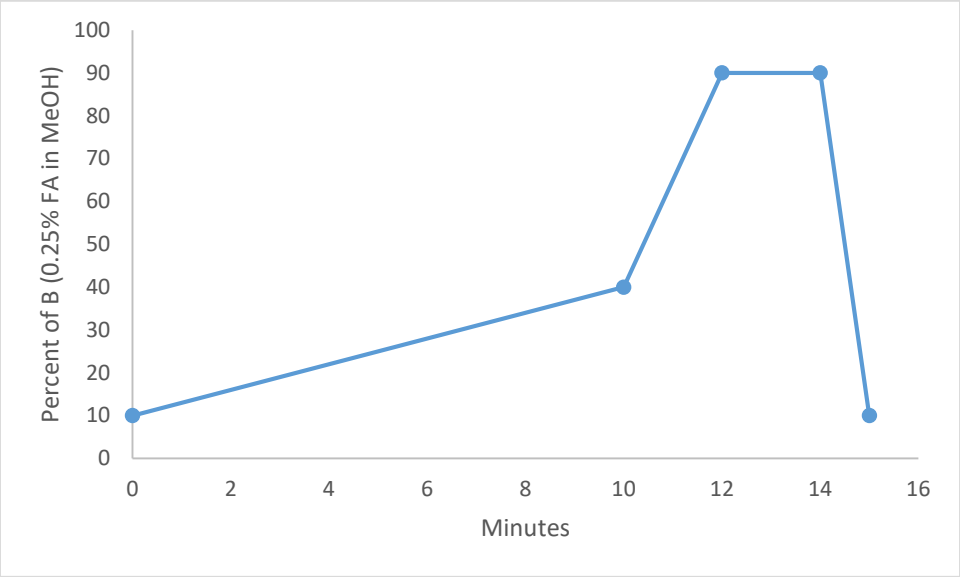


Figure 15. Water/methanol gradient for product ion studies

Solvent A is 0.25% formic acid in Milli-Q water and solvent B is 0.25% formic acid in methanol. The gradient starts from 10% B, reaches 40% B within ten minutes. The percentage of solvent B continues to increase for another two minutes and is held on at 90% B for five minutes. The percentage of B decreases from 90% to 10% within one minutes (Figure 15).

CHAPTER FOUR

DEMONSTRATION OF ANALYTICAL METHOD FOR DETECTION OF MULTICHLORINATED SPECIES IN NATURAL WATER AND WASTEWATER EFFLUENT

New pollutants are known to arise in aquatic environments worldwide continuously. This serves as motivation for the investigation of novel pollutants in natural water sources around the Chicago metropolitan area, primarily Lake Michigan and Chicago River. Water samples obtained from Stickney waste water treatment plant, one location on the shore of Lake Michigan (Buckingham Fountain), seven locations along the Chicago River (West River Park, Weed Street, Madison Street, Erie Street, Daley Park boat launch, Cicero Ave and Willow Springs boat launch) were analyzed using precursor ion scans for chlorine-containing contaminants. The most abundant and persistent chlorine containing ions were analyzed by high resolution mass spectrometry and product ion scans, and possible empirical formula assignments will be presented here.

This chapter is divided in four sections. Section 1 demonstrates the efficacy of the precursor ion method through the detection of known chlorinated pollutants, sucralose and triclosan in environmental samples. Section 2 describes the preliminary screening of chlorinated compounds detected in Stickney WWTP, Lake Michigan and Chicago River system using tandem mass spectrometry. In section 3, the discovery of a dichlorinated compound with a nominal molecular weight of 241 Daltons is detected and characterized in treated waste water

and at several locations around the Chicago River. In section 4, the most abundant and persistent trichlorinated compound with a nominal molecular weight of 246.4 daltons, detected in five out of seven locations sampled around the Chicago River, is characterized.

Initial Demonstration of Method

Sucralose and Triclosan

Sucralose ($C_{12}H_{19}Cl_3O_8$), a trichloro compound, is widely used as an artificial sweetener in products such as Splenda. Sucralose is not metabolized by human body, so it is discharged into waste water or natural water with its molecular structure intact. Therefore, it should be detected in water samples using the precursor ion scan method described here. This compound is detected in waste water effluent using our precursor ion scan method. Extracted ion chromatograms are shown below in Figure 16. Sucralose is a molecule that contains three chlorine atoms. The retention time is 7.4 minutes when analyzed using a 30 minute water/methanol gradient described in Chapter 3. Figure 16A and 16B show chromatograms of the m/z 395->35 and m/z 396 (C13 ion)->35 transitions, respectively. The ion at m/z 395 is the monoisotopic ion containing three ^{35}Cl atoms, and m/z 397 (Figure 16C), m/z 399 (Figure 16E) contain two ^{35}Cl, one ^{37}Cl and one ^{35}Cl, two ^{37}Cl atoms, respectively. Although no adverse effects of sucralose in the environment has been documented so far, the fact that it accumulates in natural water sources has caused concern [13]. We have detected sucralose in water sampled at Madison Street (Water Taxi Pier), Daley Park boat launch in Sanitary Ship Canal (SSC), Cicero Ave (SSC) and the Willow Spring boat launch along the Chicago River (SSC) (Appendix C). We have found sucralose in Lake Michigan as well [47].

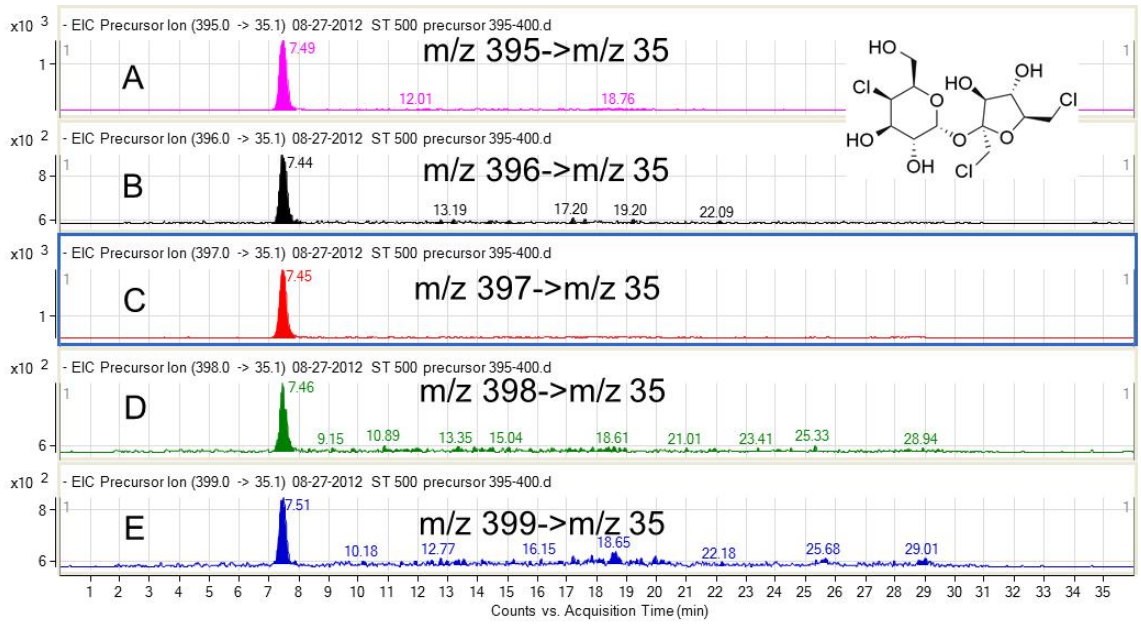


Figure 16. Extracted ion chromatograms of precursor ions that fragment to give ^{35}Cl product ions consistent with the empirical formula of sucralose extracted from wastewater effluent

Another trichloro compound, triclosan ($C_{12}H_7Cl_3O_2$), is antibacterial agent widely used in consumer products, such as soaps, toothpastes, detergents, and surgical cleaning treatments [48]. Upon disposal, despite the high percentage removal by municipal waste water treatment plants (WWTPs) from the aqueous phase, 97-98% [49], 170000-970000 kg/yr of triclosan is known to break through WWTPs and subsequently may pose serious effects on ecological water environment [50]. This antimicrobial compound may destroy microorganisms necessary to maintain a healthy aquatic environment. Therefore we expect to detect triclosan in water samples with our precursor scanning method. Extracted ion chromatograms are shown in Figure 17. The precursor ions in Figure 17 are $(M-H)^-$ deprotonated molecule ions.

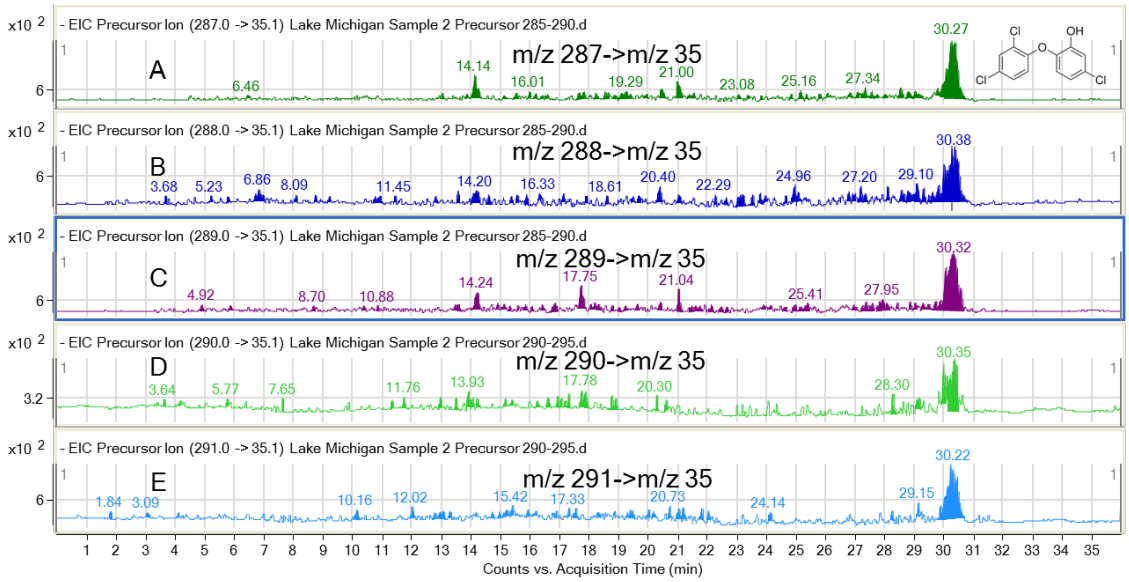


Figure 17. Extracted ion chromatograms of precursor ions that fragment to give ^{35}Cl product ions consistent with the empirical formula of triclosan from Lake Michigan water sample acquired near Buckingham fountain

As shown in Figure 17, Triclosan has a retention time of 30 minutes. Figures 17A and B show the transitions of m/z 287->35 and m/z 288 (^{13}C ion)->35 respectively. The ion at m/z 287 contains three ^{35}Cl atoms. The ion at m/z 289 contains two ^{35}Cl and one ^{37}Cl atom (Figure 17C). The ion at m/z 291 (Figure 17E) contains one ^{35}Cl and two ^{37}Cl atoms. The triclosan precursor ions in Figure 17 are $(M-H)^-$ deprotonated molecule ions.

Triclosan was also detected in Stickney waste water effluent, and near the water taxi pier at Madison Street along Chicago River. The identities of the sucralose and triclosan were confirmed by accurate mass analysis and cochromatography with standards. The results shown above suggest that precursor ion scanning of ^{35}Cl over narrow, consecutive mass ranges (five daltons) is an effective means of detecting chlorine containing contaminants in water samples. Extracted ion chromatograms are utilized to determine the numbers of chlorine atoms in the precursor ions. Precursor ion scanning over narrow mass ranges is used to analyze natural water

samples taken from Lake Michigan and Chicago River for unknown chlorinated compounds as described below.

Chlorinated Compounds Detected in Stickney WWTP, Lake Michigan, and the Chicago River System

Over the course of this study we have analyzed two samples from the Stickney WWTP acquired on different dates. Seven locations along the Chicago River were also sampled. Samples were acquired fifteen minutes apart at the same site (Madison Street water taxi pier and at Buckingham Fountain) for the purpose of assessing fluctuations in concentration over a short interval of time. Additional water samples were acquired for the purpose of conducting product ion studies (Erie, Daley and Cicero) of ions detected in the precursor ion scans. These product ion studies are discussed in more detail in Chapter 6. The sample date, location, and numbers of chlorinated compounds/ions detected in the corresponding samples by precursor ion scanning are presented in Table 5 below. The number of chlorinated ions is greater than the number of chlorinated compounds because of the ^{37}Cl peaks (compound sucralose vs. m/z 395, 397, and 399). Retention times and m/z values of all chlorinated ions detected (the signal to noise ratio greater than 3:1) in this study are listed in the Appendices.

In this section, chlorinated compounds found in Stickney waste water effluent will be discussed first; chlorinated compounds detected in Lake Michigan will be reviewed second; and last, chlorinated compounds detected in Chicago River system will be discussed briefly.

Table 5. Sampling Sites, Dates, and Numbers of Chlorinated Ions/Compounds Detected in Precursor Ion Analyses

Sampling Sites	Sampling Dates	Number of Chlorinated Ions Detected	Number of Chlorinated Compounds Detected
Stickney WWTP	08/09/2012	61	40
Stickney WWTP	01/17/2013	41	30
Lake Michigan Buckingham	05/09/2013	37	29
Lake Michigan Buckingham	06/11/2013 (2)	45, 52	40, 45
Chicago River West River Park	06/27/2013	51	48
Chicago River Weed Street	06/27/2013	54	51
Chicago River Madison Street	05/09/2013	34	34
Chicago River Madison Street	05/30/2013 (2)	31, 39	29, 36
Chicago River Daley Park	07/11/2013	47	38
Chicago River Willow Spring	07/11/2013	25	25
Chicago River Erie Street	08/14/2013	32	29
Chicago River Cicero Ave	08/21/2013	27	25

Chlorinated Compounds Found in Waste Water Effluent

Forty compounds containing at least one chlorine atom were detected (including Sucralose and Triclosan) in waste water effluent obtained on Aug 9th, 2012 from the Stickney WWTP (Appendix A) and thirty compounds containing at least one chlorine atom were detected in waste water effluent obtained on Jan 17th, 2013. Stickney does not treat wastewater with hypochlorite. Therefore the chlorinated compounds detected in this sample must come from other sources, such as PCPPs (personal care products and pharmaceuticals) that are not removed during the water treatment process.

We analyzed a four-hundred Dalton mass range (m/z 200 to m/z 600) with eighty total injections. This mass range was chosen because a recent study of chlorinated natural organic matter (NOM), suggested that over 95% of 1007 chlorinated organic compounds detected formed molecule ions in this mass range [32].

LC/MS/MS analysis suggests that there are 15 different chlorinated compounds detected in both Stickney samples. The masses of the monoisotopic molecular anions and their retention times are given in Table 6 below.

Table 6. Chlorine Containing Compounds Detected in Both Waste Water Effluents from Stickney

m/z	Retention Time (minutes)
211	17.1
231	19.7
245/247/249	22.0
251	19.6
257	9.65
258	25.8
265	20.5
287/289/291	30.0
296.9	23.9
330.1	27.1
367/369/371	30.4
395/397/399	7.50
410.1	27.2
431/433/435	7.40
441/443	7.74

The data in Table 6 indicate there are several ions having similar retention times that contain more than one chlorine atoms. For example, if a molecule contains two chlorine atoms, there are three combinations of these two chlorine atoms: $^{35}Cl^{35}Cl$, $^{35}Cl^{37}Cl$, and $^{37}Cl^{37}Cl$. Therefore, in the precursor ion scan analysis, two peaks could be observed as the precursor ions of ^{35}Cl and have the same retention times. In Table 6, m/z 441 and m/z 443 both have retention time 7.74 min, which indicates that m/z 441/443 contain two chlorine atoms.

Chlorinated Compounds Found in Lake Michigan

Lake Michigan is the primary drinking water source of the Chicago metropolitan area, thus the quality of its water deserves special attention. Water samples were acquired from Lake Michigan shoreline and analyzed for chlorine-containing compounds using the precursor ion method described above.

Water samples were acquired at Buckingham Fountain (Figure 12) on May 9th, 2013 and June 11th, 2013. Two samples were taken on June 11th, 2013 within a fifteen minute interval at Buckingham Fountain location to see how the chlorinated ion profile might change as a function of time, given as the Lake is not expected to be a homogenous solution and that concentrations will fluctuate with time. A list of chlorinated ions detected in the precursor ion analysis in all three of the Buckingham Fountain samples is given in Appendix B.

In order to show whether the chlorine compounds in Lake Michigan samples vary with time, the chlorine compounds detected in both of the June 11th, 2013 samples are compared and the thirteen common compounds are shown below in Table 7.

It can be seen from Table 7, that both Triclosan and Sucralose were detected in the two Lake Michigan samples taken from Buckingham Fountain within fifteen minutes interval. In the forty chlorine containing compounds found in Buckingham Fountain sample 1 and forty five chlorine containing compounds found in Buckingham Fountain sample 2, which was obtained after fifteen minutes, only thirteen common ions were detected. The results indicate that the number of chlorine containing compounds detected may vary a great deal after sampling the same sites within fifteen minutes. Our results suggest that many of the chlorinated species we detect have short lifetimes in natural water.

Table 7. Chlorine Compounds Detected in Both Jun 11th, 2013 Lake Michigan Samples Obtained at Buckingham Fountain within a Fifteen Minute Interval

m/z	Retention Time
333	22.5
401	22.5
399/401	25.8
331	20.2
377	20.1
417/419/421	29.4
354	19.4
305	21.4
287/289/291	30.2
395	7.75
591	21.4
340	17.8
200	31.3

Chlorinated Compounds Found in Chicago River

Seven different sites along the Chicago River were sampled over the course of a year between June 2012 and August 2013 and analyzed for chlorine-containing species. The Madison street pier was sampled several times, twice within a fifteen minute interval. The ions most frequently detected (based on m/z value and retention time) at different sampling sites are analyzed further to determine their structures in order that questions regarding their potential human or environmental toxicity may be addressed.

All compounds detected in the Chicago River that contain at least one chlorine atoms are tabulated in Appendix C. Madison street samples were obtained on May 9th, 2013 and twice on May 30th, 2013 (within fifteen minute interval). A comparison of chlorinated compounds found in the two samples obtained on May 30th, 2013 are listed individually in Table 8.

The result of precursor ion study for Chicago River samples taken at different times indicate that many of the chlorinated species we detected may have degraded by O_2, bacteria in natural water. In Madison street water taxi pier sample 1 obtained on May 30th, 2013, twenty

nine chlorinated compounds were detected, whereas in sample 2 (obtained within fifteen minute interval), thirty six chlorinated compounds were detected (Appendix C). Comparison of the ions detected in these two Madison street water taxi pier samples shows that there are only eleven common ions present. Therefore, chlorinated compounds vary significantly between different times, even at short time intervals like fifteen minutes.

Table 8. Chlorine Compounds Detected in Both May 30th, 2013 Chicago River Samples Obtained at Madison Street Water Taxi Pier within Fifteen Minute Interval

m/z	Retention Time (Minutes)
226.9	14.5
245	21.3
250	19.8
220.1	13.5
228	7.51
233	15.1
203	17.3
265	21.9
205	14.6
202	7.47
421	16.9

We also compared numbers of chlorinated compounds detected in two Stickney waste water effluent samples, those in two Lake Michigan samples obtained near Buckingham Fountain, and in two Chicago River samples obtained at Madison Street water taxi pier. The number of common chlorinated compounds are listed in Table 9.

So in Table 9, the number of chlorinated compounds detected in two Stickney waste water treatment effluent samples, two Lake Michigan samples obtained at the same location and different dates and two Chicago River samples obtained at the same location and different dates are compared.

The cross comparisons of ions detected at different sites show that the two samplings conducted at the Stickney wastewater treatment plant yielded the largest number of common

(m/z value and retention time) with the numbers of same chlorinated compounds as Chicago River sample (May 30th, 2013), eight and eleven respectively. These chlorinated compounds those had not been removed by the waste water treatment process will enter Chicago River and may pose adverse ecological effects.

Table 9. Comparison of Chlorinated Compounds Detected in Two Stickney Waste Water Effluent Samples, Two Samples Obtained at Lake Michigan Buckingham Fountain and Two Samples Obtained at Chicago River Madison Street Water Taxi Pier

Number of chlorine compounds	Stickney 08/09/12	Stickney 01/17/13	Lake 05/09/13	Lake 06/11/13	River 05/09/13	River 05/30/13
Stickney 08/09/12	_____	15	2	7	5	11
Stickney 01/17/13	15	_____	7	5	5	8
Lake 05/09/13	2	7	_____	8	8	2
Lake 06/11/13	7	5	8	_____	6	3
River 05/09/13	5	5	8	6	_____	7
River 05/30/13	11	8	2	3	7	_____

Compound Identification Based on Average Molecular Mass and Number of Chlorines

It is possible to use the nominal ion masses and number of chlorines to identify the chlorinated compounds detected in the LC/MS/MS analyses. The number of chlorines and masses of the $(M-H)^-$ ions detected may be used to estimate an average molecular weight that can be used to search a database like Scifinder Scholar to identify these compounds. When one consider the isotopic abundance associated with the number of chlorines in a given compound, it is possible to estimate an average molecular mass to four significant digits. In Table 6, the compound that produced the monochlorinated, monoisotopic ion at m/z 211 will likely have an average molecular weight (211×75% + 213×25% +1, peak ratio 3:1) that is close to 212.5

grams/mole. The dichlorinated compound at m/z 399/401 with a retention time of 25.8 minutes will have an average molecular weight (399×56.25% + 401×37.5% + 403×6.25% +1, peak ratio 9:6:1) close to 401.The trichlorinated species (Table 7) with ions at m/z 417/419/421 is expected to have an average molecular weight (417×42% + 419×42% + 421×14% + 423× 2% +1, peak ratio 27:27:9:1) close to 419.5. When these average molecular weights are used to search SciFinder, a mass variance of ± 0.25 daltons was used. When these search parameters are used for the average molecular weights suggested by the ions at m/z 287/289/291 in Tables 6 and 7 and m/z 395/397/399 in Table 6, sucralose and triclosan are returned as the most probable compounds when potential compounds are ranked based on their possible number of commercial sources. Therefore such a search strategy has the potential to be an accurate way of identifying compounds based on their LC/MS/MS characteristics.

The compound with an average molecular weight of 212.5 daltons (Table 6, Rt 17.1 minutes) returns 16,035 compounds (search date 1/10/17). The most probable compound based on our search criterion would be 3-Chlorobenzo[*b*]thiophene-2-carboxylic acid (CAS 21121-22-3, 149 possible sources). The compound with an average molecular mass of 401.0 daltons (m/z 399/401, Table 7, Rt 25.8 minutes) returns 1,378 possible compounds with 2',7'-Dichloro-3',6'-dihydroxy-Spiro[isobenzofuran-1(3*H*),9'-[9*H*]xanthen]-3-one (CAS 76-54-0) with 43 possible commercial sources. The compound with an average molecular weight of 419.5 daltons gave 276 possible compounds with 1-[2,5-bis(2,2,2-trifluoroethoxy)phenyl]-2,2,2-trichloro-ethanone (CAS 76784-42-4) with 11 possible commercial sources.

These results suggest/verify that more information will be required to identify unknown chlorinated compounds in aqueous environments, particular those with molecular masses between 200 and 300 daltons with one or two chlorines. Accurate mass and product ion data will

need to be used to narrow down the potential identities of an unknown chlorinated compound, even if the physical properties of a compound are recorded in a database.

Characterization of Persistent Dichlorinated Pollutants

In this section, we describe the identification of a persistent dichlorinated pollutant that shows a consistent deprotonated molecule at m/z 241 eluting between five and six minutes depending on the gradient used. This compound has been detected on three occasions in Stickney wastewater effluent (Aug 9th, 2012, Jan 17th, 2013 and Aug 7th, 2014), and at three sites (Erie Street Park, Daley Boat Launch along South Sanitary Canal, and Cicero Ave along South Sanitary Canal) along the Chicago River at different times in three consecutive years. This compound was present in Erie Street sample taken on Aug 14th, 2013, Daley Boat Launch sample taken on July 11th, 2013 and Cicero Ave sample taken on Aug 21th, 2013 respectively. In 2014, this dichlorinated compound was detected in Erie Street sample obtained on July 3rd, 2014, Daley Boat Launch sample obtained on July 9th, 2014 and Cicero Ave sample obtained on July 11th, 2014 respectively. In 2015, the dichlorinated molecule was detected in Erie Street sample obtained on July 23rd, 2015, Daley Boat Launch sample obtained on July 25th, 2015 and Cicero Ave sample obtained on July 24th, 2015 respectively. Thus, this compound has been detected in both natural water and treated wastewater effluent, so it has the potential to bioaccumulate.

Precursor Ion Analysis of a Persistent Dichlorinated Pollutant Extracted from Different Water Sources

The precursor ion analysis of a Stickney effluent sample obtained on Aug 9th, 2012 produced abundant ions in the m/z 240 to m/z 245 mass range. The extracted ion chromatograms are shown in Figure 18. Several transitions are observed to have the same retention time. The ions at m/z 241 and m/z 242 (C13 peak) have two chlorine-35 atoms (Figure 18A and B), while

the ions at m/z 243 and m/z 244 (C13 peak) have one chlorine-35 and one chlorine-37 atom (Figure 18C and D). The ion at m/z 245, contains only chlorine-37 atoms, so it is not detected in this experiment at the same retention time as m/z 241-244. The m/z 245 ion seen in Figure 18E is formed from a different chlorinated compound.

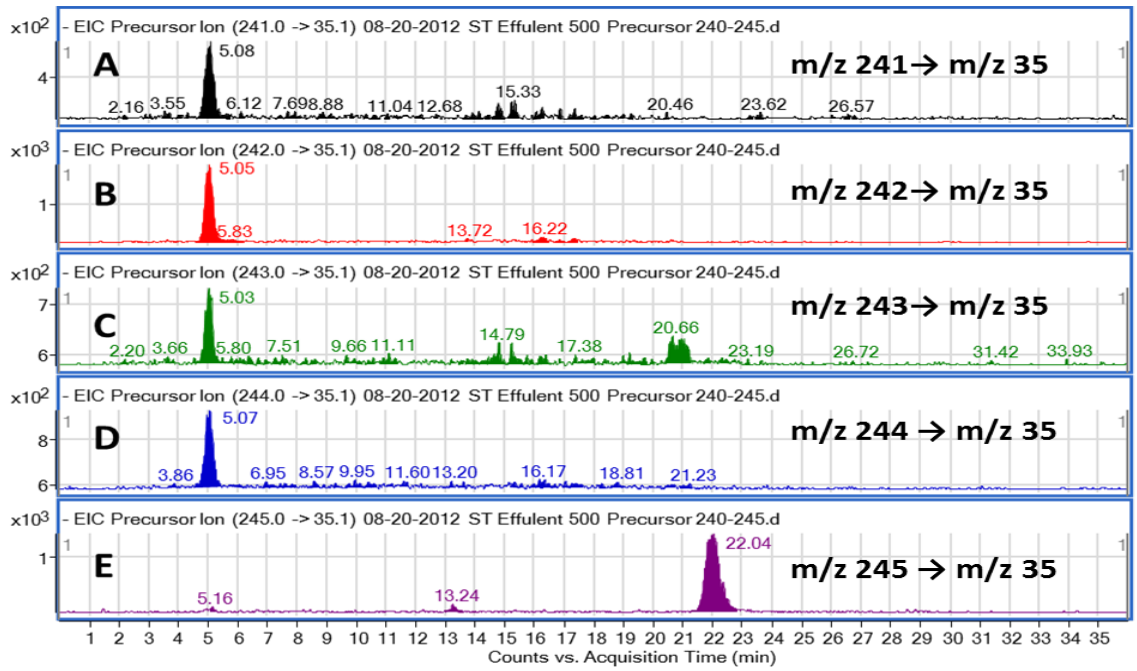


Figure 18. Extracted ion chromatograms derived from the precursor ion analysis of a Stickney wastewater effluent sample (Aug 9th, 2012) acquired over a m/z 240 to m/z 245 mass range

This dichlorinated species eluting with a retention time of five minutes in the initial precursor ion analyses has been detected in water samples acquired at Stickney wastewater treatment plant (effluent) and was shown in Figure 18 above, the Chicago River at Erie Street and the Chicago Sanitary and Shipping Canals (CSSC-Figure 12) at Daley boat launch and Cicero Ave on three occasions over the last three years (Figure 19). The dichlorinated species has not been detected at either Lake Michigan sampling site along the coast specified in Figure 12 to date.

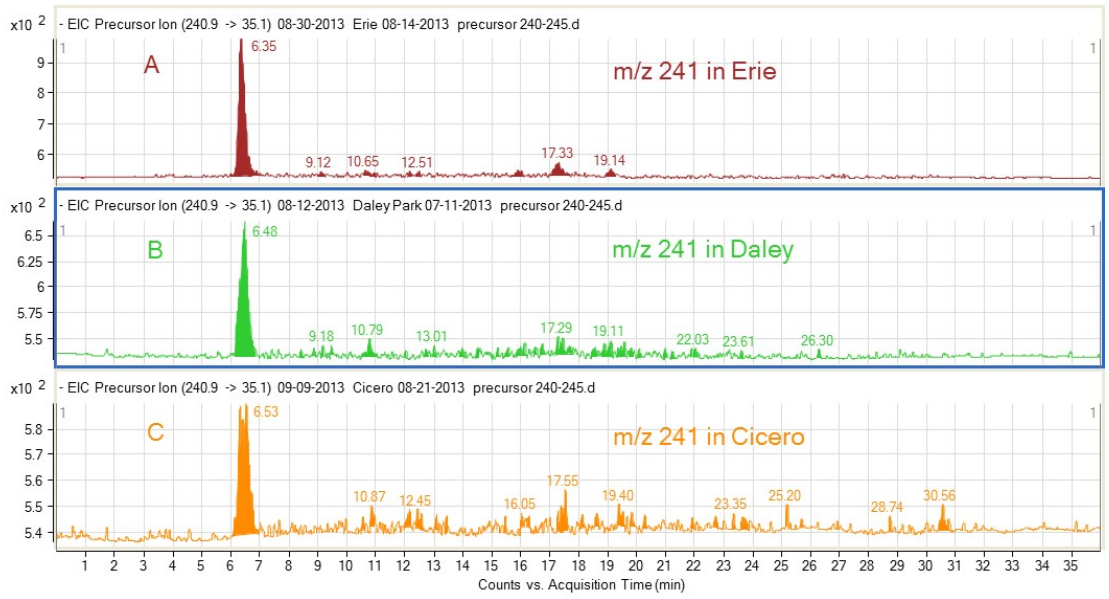


Figure 19. Extracted ion chromatograms derived from the precursor ion analyses of Chicago River water samples acquired at (A) Erie Street Park (B) Daley Boat Launch along SSC and (C) Cicero Ave along SSC over a m/z 240 to m/z 245 mass range

The retention time of m/z 241 in Stickney effluent sample (5 minutes) does not match exactly with those in Erie (6.4 minutes), Daley Park and Cicero Ave samples (6.5 minutes). The reason is that Stickney effluent precursor ion test was performed in 2012 using a different gradient than that used for Erie Street, Daley boat launch and Cicero Ave samples' precursor ion tests performed in 2013 and thereafter. The gradient used for the analysis of natural water samples was more aqueous than the gradient used for the analysis of effluent water and so the retention time of the chlorinated species is longer (6.5 vs. 5.0 minutes).

These precursor ion analyses indicate the apparent molecular mass, the retention time, and number of chlorine atoms in the molecule detected. The structural verification of this dichlorinated molecule is described below.

Accurate Mass Analysis of the Dichlorinated Pollutant Having a Molecule Ion at m/z 241

Accurate mass data was acquired to determine the empirical formula of the particular chlorinated compound with an apparent molecule ion at m/z 241. Time-of-flight full-scan mass spectra showing the m/z 241-m/z 245 region eluting at approximately six minutes from two different water samples are shown in Figure 20. The **measured** masses are consistent with the empirical formula $C_6H_3Cl_2SO_4$. The measured masses of the three most abundant isotopic masses presented in Figure 20 A and B are within 4 ppm of the theoretical mass of $C_6H_3Cl_2SO_4$. The relative abundances of the ions at m/z 240.9, m/z 242.9, and m/z 244.9 (9:6:1) are consistent with empirical formula containing two chlorine atoms as well. The valences of the atoms in the empirical formula (rings-plus-double bonds analysis) suggests that these ions are $[M-H]^-$ ions derived from a compound with the empirical formula $C_6H_4Cl_2SO_4$ containing four elements of unsaturation.

The data shown in Figure 21 indicates that the empirical formula whose theoretical mass closest to the measured mass (plus a hydrogen atom) of the consistent empirical formula with two chlorine atoms contains four elements of unsaturation. This suggests the presence of an aromatic ring, which is supported by the product ion spectra discussed below.

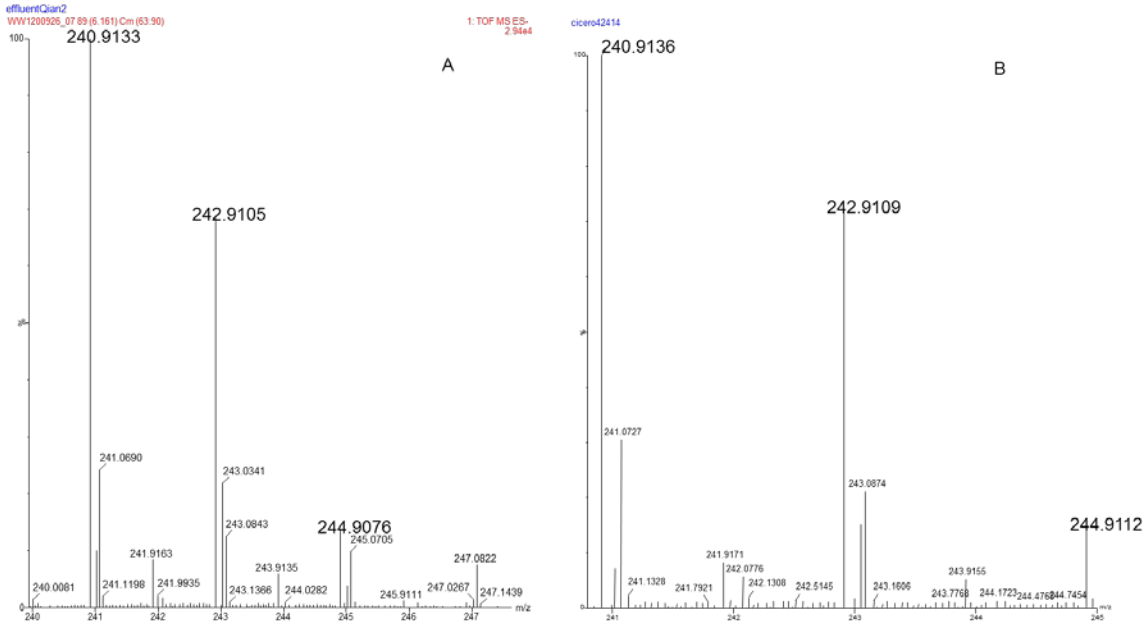


Figure 20. Partial Q-TOF mass spectra of chlorinated ions at m/z 241, m/z 243, and m/z 245 indicated by accurate mass analysis to contain two chlorine atoms obtained from A Stickney waste water effluent and B Chicago River at Cicero Ave

Experimental monoisotopic mass: 241.9211

MF range: C0-30H0-60N0-5O0-10F0-3Cl2 S1

Search MF

Results:

MF	Monoisotopic mass	PPM	mDa	Unsaturation
$C_6H_4Cl_2O_4S$	241.920734976	-1.509	-0.365	4
$C_3H_5Cl_2FO_5S$	241.9218778547	3.215	0.778	0
$C_7Cl_2N_4S$	241.9220723889	4.019	0.972	9
$C_4HCl_2FN_4OS$	241.9232152676	8.744	2.115	5
$C_4H_3Cl_2F_3O_2S$	241.9182903858	-11.614	-2.810	1
$CH_2Cl_2F_2N_4O_2S$	241.9243581462	13.468	3.258	1
$C_7H_2Cl_2F_2OS$	241.9171475072	-16.338	-3.952	5
$CH_4Cl_2N_2O_6S$	241.9167122249	-18.138	-4.388	0
$C_{10}HCl_2FS$	241.9160046286	-21.063	-5.095	9
$H_4Cl_2N_4O_5S$	241.927945615	28.296	6.846	0
$C_6H_2Cl_2F_2N_2S$	241.9283808974	30.095	7.281	5

Figure 21. Screen shot of potential empirical formulas and their deviation from the molecular weight suggested by the measured m/z value from Stickney effluent acquired on Aug 9th, 2012 http://www.chemcalc.org/web/mm_description

Product Ion Spectra of the Dichlorinated Pollutant

Product ion spectra of the $[M-H]^-$ ion at m/z 241 ion generated from different water samples were acquired and compared to gain some insight into the structure of the unknown chlorinated pollutant. Product ion spectra of the m/z 240.9 acquired using the triple quadrupole mass spectrometer suggest dichlorinated compounds isolated from different water samples have the same structure (Figure 22). The spectra of m/z 240.9 ion isolated from different water samples indicate that this ion is suggested to be an aromatic sulfonic acid (Figure 22).

Product ion spectra are shown for the m/z 241 ion extracted from the wastewater effluent sample (Figure 22A) and a sample taken from Daley boat launch along the Sanitary and Shipping Canal (Figure 22B). Product ion spectra from these two samples were chosen for Figure 22 because these two samples provided the most abundant m/z 241 ions in the original precursor ion scans (scanning in the m/z 240 to m/z 245 mass range). Both product ion spectra show the same five major fragment ions at similar relative abundances, suggesting that the compounds extracted from the two different water samples have the same chemical structure. In Figure 22, these five fragment ions are m/z 35 (chloride ion), m/z 80 (the SO_3^- ion), m/z 113 (formed by the neutral losses of CO, HCl, and SO_2), m/z 177 (formed by the neutral loss of SO_2), and m/z 205 (formed by the neutral loss of $H^{35}Cl$). There is no apparent fragmentation pathway that involves the breaking of a carbon-carbon bond (a series of ions that are 14-dalton apart). This is consistent with four unsaturation (Figure 21), suggesting the presence of a benzene ring. This observation, taken with the fragmentation pathways specified in the product ion spectra; suggest a substituted benzene ring structure. This product ion mass at m/z 80 is consistent with an SO_3^-, which is a signature for a sulfonic acid group.

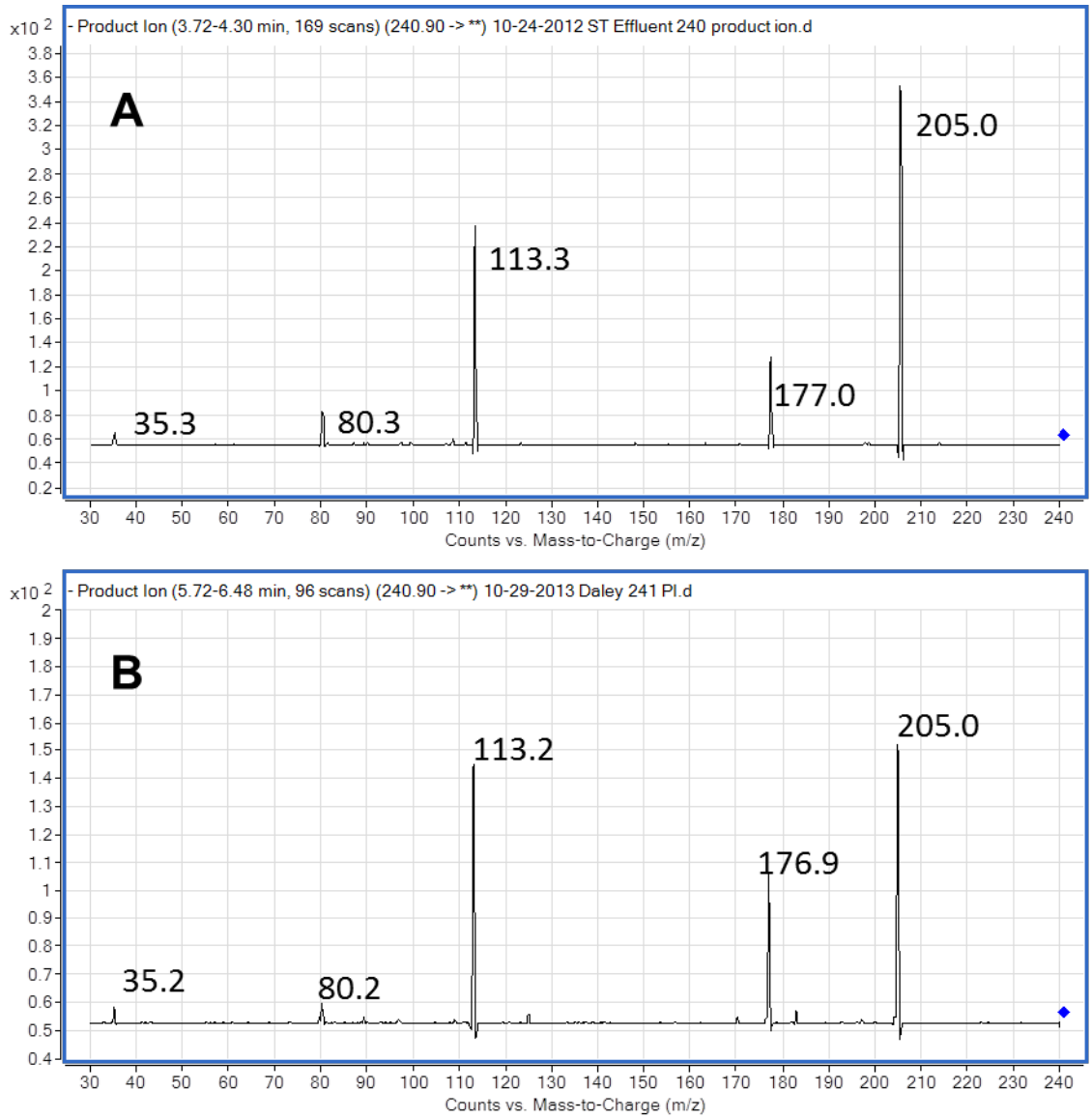


Figure 22. Product ion mass spectra of m/z 240.9 ion from a wastewater effluent sample acquired on a triple quadrupole mass spectrometer A extracted from wastewater treatment plant effluent and B a Chicago River water sample taken from the Daley Park along Sanitary and Shipping Canal

The loss of water is consistent with the presence of a phenol. The product ion spectrum suggests that this compound is a dichlorobenzene phenol sulfonic acid ($C_6H_4Cl_2O_4S$). A series of hydroxy dichlorobenzene sulfonic acids were synthesized to determine the final structure of this compound and the characterization will be discussed in Chapter 5.

Analysis of a Trichlorinated Compound with a Molecular Ion of m/z 245

The second persistent chlorinated compound that attracted attention from the precursor ion scan of ^{35}Cl result is the trichlorinated compound having molecular ion of m/z 245 and are present in five out of the seven sites along the Chicago River sampled (West River Park on June 27th, 2013, Weed Street on June 27th, 2013, Erie Street Park on Aug 14th, 2013, Madison Street Boat Launch on May 30th, 2013, Willow Spring Boat Launch on July 11th, 2013). The same approach of identification will be utilized here; I will discuss how chromatography is used to verify the molecular weight and number of chlorine atoms first. This is followed by a discussion of the empirical formula and structural characteristics implied by the full scan mass spectra. Third, product ion spectra will be used to suggest a structure as well.

Precursor Ion Analysis of a Persistent Trichlorinated Pollutant Extracted from Different Water Sources

The chromatograms will be presented below belong to the precursor ion scan for ^{35}Cl conducted over the m/z 245-m/z 250 mass range in the analysis of a wastewater effluent sample obtained on Aug 9th, 2012. The extracted ion chromatograms (Figure 23A-23E) taken together suggest the presence of a compound with three chlorine atoms. Five transitions involving m/z 245-m/z 249 to m/z 35 are suggested to have the same retention time. The ions at m/z 245 and m/z 246 (C13 peak) have three chlorine-35 atoms (Figure 23A and 23B), while the ions at m/z 247 and m/z 248 (C13 peak) have two chlorine-35 and one chlorine-37 atom (Figure 23C and D). The ion at m/z 249, contains only one chlorine-35 and two chlorine-37 atoms (Figure 23E).

The m/z 245 ion detected in five locations of Chicago River as stated above is indicated to have the same empirical formula by accurate mass analysis and product ion spectra.

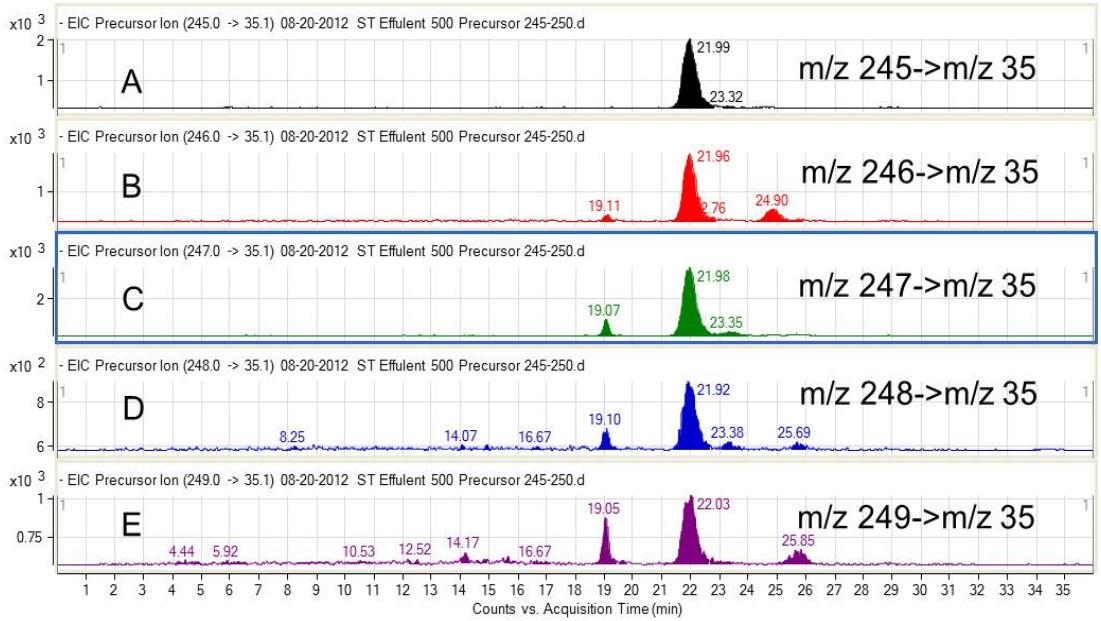


Figure 23. Extracted ion chromatograms derived from the precursor ion analyses of Stickney effluent sample acquired on Aug, 9th, 2012 over a m/z 245 to m/z 250 mass range

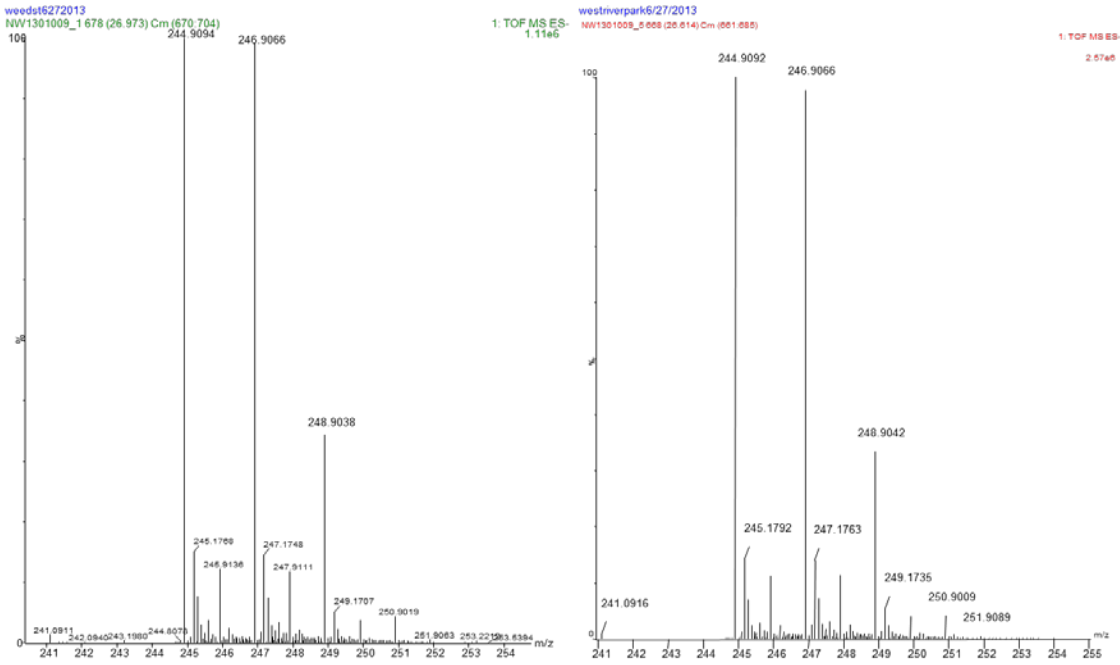


Figure 24. Q-TOF mass spectra of chlorinated ions at m/z 244.9/246.9/248.9 accurate mass analysis to contain three chlorine atoms obtained from (A) Weed Street and (B) West River Park

Molecular Ion Formation Characteristics and Accurate Mass Analysis of the Trichlorinated Pollutant and Standard Compounds

The high resolution mass spectra of this trichlorinated pollutant are shown in Figure 24. The relative abundance ratios of the ions at m/z 245, 247, 249, and 251 are approximately 27:27:9:1 in both samples shown, consistent with the isotope ratios expected for a trichlorinated compound.

Accurate mass analysis suggests a molecule containing three chlorine atoms, and the best fit is consistent with an empirical formula of $C_6H_6Cl_3PO_2$ (Figure 25) with an error of 0.4 ppm. Thus, the compound might have an empirical formula of $C_6H_6Cl_3PO_2$ if the molecular ions observed in the Figure 24 mass spectra are formed by deprotonation ($[M-H]^-$).

However, a Scifinder empirical formula search suggests there is no known compound with that empirical formula ($C_6H_6Cl_3PO_2$). The next possibility is that the ions formed in the Figure 24 electrospray spectra were radical anions ($C_6H_5Cl_3PO_2^{-\cdot}$) formed by electron attachment. The ions formed would have the same empirical formula as the compound, $C_6H_5Cl_3PO_2$. Again, a molecular formula search on SciFinder did not provide any compounds with this molecular formula. We then made the hypothesis that this compound had a quinone structure (or a structure that would ionize like a quinone) [36]. Such an ionization mechanism assumes that the compound would be reduced during the electrospray process and add a proton to form a $[M+H]^-$ ion. This is demonstrated in scheme 4.1 below [37].

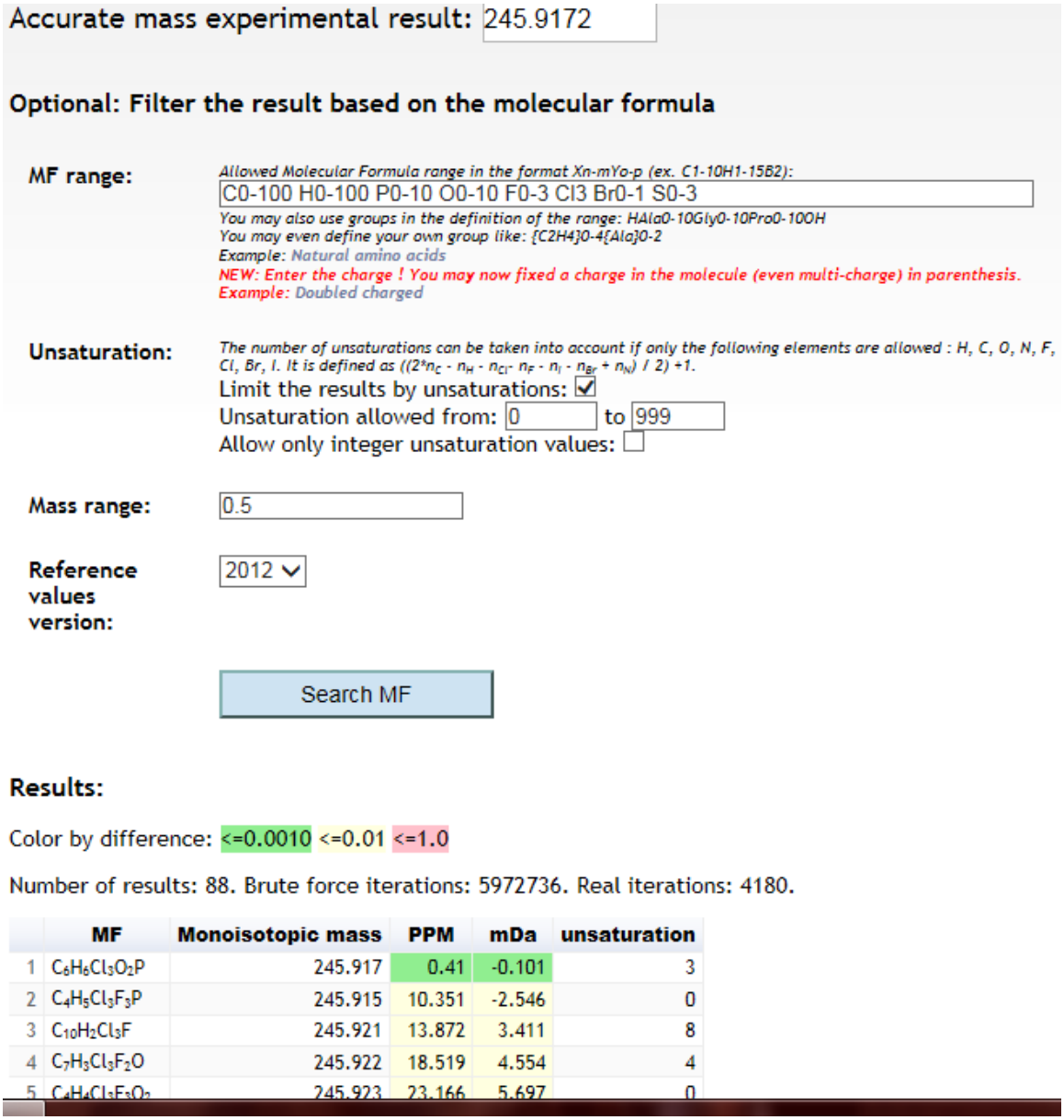
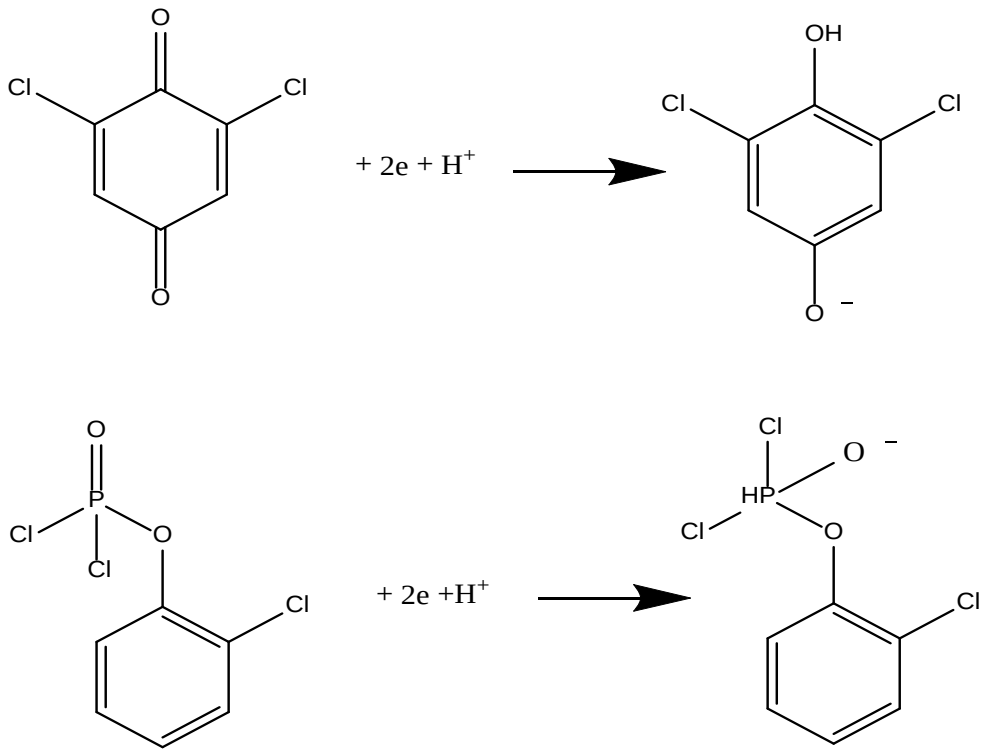


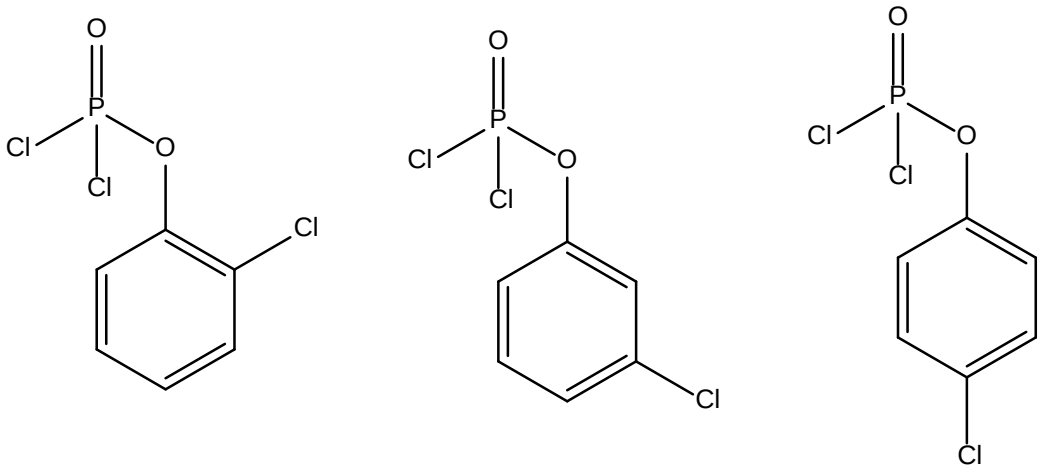
Figure 25. Potential empirical formulas and their deviation from the molecular weight suggested by the measured m/z 244.9094 value from Chicago River at Weed Street acquired on June, 27th 2013. http://www.chemcalc.org/mf_finder/mfFinder_em_new



Scheme 1. Ionization reaction of dichloroquinone and chlorophenyl dichloro phosphate

We searched the SciFinder for compounds having a molecular formula of $C_6H_4Cl_3PO_2$ and three compounds were found; 2-chlorophenyl dichlorophosphate, 3-chlorophenyl dichlorophosphate and 4-chlorophenyl dichlorophosphate (Figure 26).

The meta-chloro isomer (Figure 26B) is not available commercially. Both ortho (Figure 26A) and para (Figure 26C) chloro isomers are available commercially so these compounds were analyzed by electrospray ionization to see if these ion formation characteristics were similar to that of a haloquinone. The para isomer (Figure 26C) did produce a mass spectrum under the same conditions as the environmental samples shown in Figure 24. The ortho (Figure 26A) isomer produced a mass spectrum formed by the attachment of a proton to give the mass spectrum in Figure 27.



A B C

Figure 26. Suggested structures of compounds that have formula $C_6H_4Cl_3O_2P$. (A) 2-chlorophenyl phosphorodichloridate (B) 3-chlorophenyl phosphorodichloridate and (C) 4-chlorophenyl phosphorodichloridate

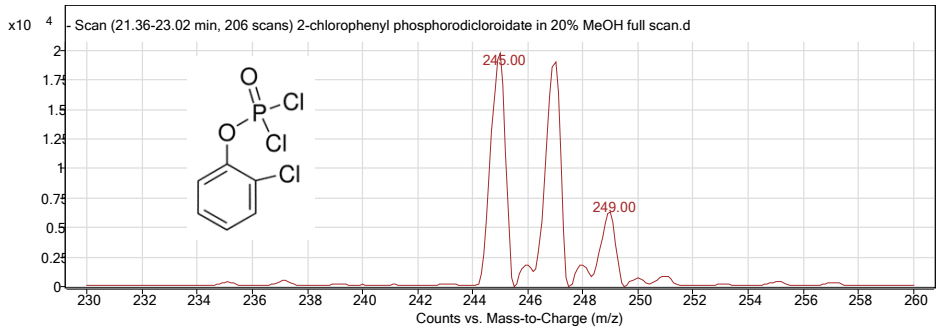


Figure 27. Full scan spectrum of 2-chlorophenyl phosphorodichloridate acquired by triple-quadrupole

Conceivably, 2-chlorophenyl phosphorodichloridate may form $[M+H]^-$ ion by two steps: first, reduction of P=O group to HP-OH forming the [M+2H] intermediate, which undergoes rapid deprotonation to produce $[M+H]^-$. This is consistent with the observation of $[M+H]^-$ ion derived from the trichlorinated compound as the most abundant ion from negative ESI in acidic solutions. We also calculate the monoisotopic mass of $C_6H_4Cl_3PO_2$, the proposed empirical formula of 2-chlorophenyl phosphorodichloridate, 243.9014. Assume $[M+H]^-$ is correct, the exact mass of $[M+H]^-$ would be 243.9014+1.0078=244.9092. In the high resolution mass

spectrometry obtained (Figure 24), m/z 244.9092 was observed and matched exactly with the calculated exact mass.

These results suggest that 2-chlorophenyl phosphorodichloridate may be the identity of the trichlorinated pollutant isolated from the Chicago River water samples. The product ion spectra of the environmental sample and standard are discussed in the next section.

Product Ion Spectra of Trichlorinated Pollutant and 2-Chlorophenyl Phosphorodichloridate Standard Compound

Product ion spectra of the 2-chlorophenyl phosphorodichloridate standard and the trichlorinated compound isolated from the environmental water samples eluting at 22 minutes were acquired for the purpose of assessing whether or not the standard might have the same structures. Product ion spectra of two environmental samples and the standard are shown in Figure 28. The ion formation characteristics of all three m/z 245 ions shown in Figure 28 appear to be the same, suggesting that both environmental samples and the 2-chlorophenyl phosphorodichloridate standard have the same structures. m/z 209.8 is formed by the loss of one chlorine atom, m/z 182 is formed by the losses of one chlorine atom and CO; m/z 175 is formed by the losses of two chlorine atoms; m/z 147 is formed by the successive losses of two chlorine atoms and one CO; and m/z 112 is formed by the losses of three chlorine atoms and one CO; and m/z 35 is the chloride ion.

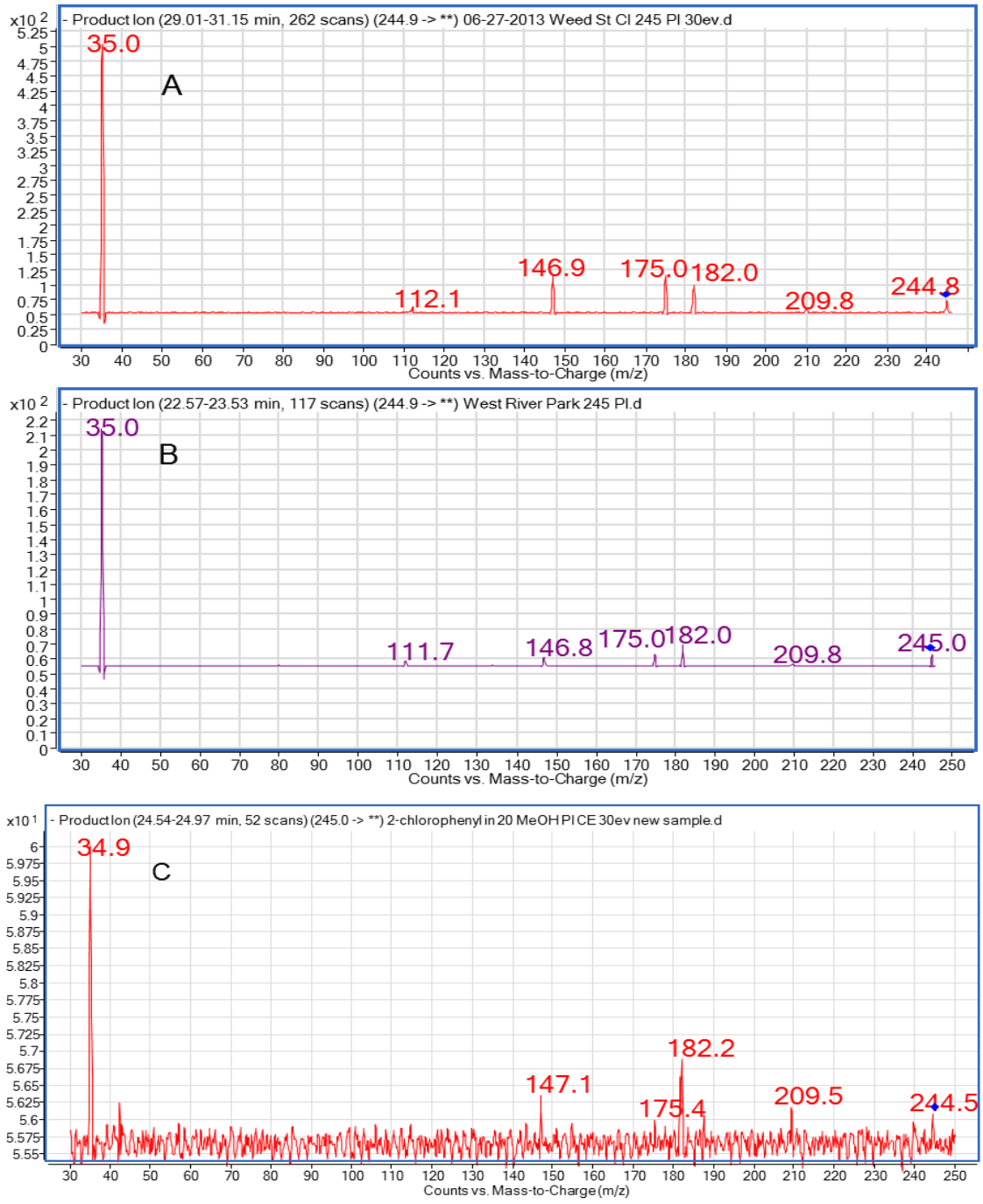


Figure 28. Product ion mass spectra (30 eV collision energy) of m/z 244.9 ion (A) from a Chicago River water sample collected at the Weed Street access point and (B) from a Chicago River water sample collected at the West River Park and (C) 2-Chlorophenyl dichlorophosphate standard eluting at 22 minutes

Possible Sources and Fate of Chlorophenyl Dichlorophosphates

Chlorophenyl dichlorophosphates are primarily used as synthetic intermediates for antimicrobial agents [38, 39]. The relatively low cost of large quantities of the ortho- and para-isomers (approximately $2/gram) suggests that excess reagent might be easily discarded [40]. The biggest question is how would a compound with this structure persist in an aquatic environment? It is generally accepted that compounds with P-Cl bonds are readily hydrolyzed when exposed to water. The 2-Chloro isomer is a liquid at ambient conditions and may enter the environment as part of an oil emulsion that may enhance its lifetime in natural water. We collect water samples from the surface of the River (or Lake Michigan). It is logical to assume that such an oil emulsion may be concentrated on the surface of the water. The lifetime of compounds like chlorophenyl dichlorophosphates may be enhanced in an aquatic environment if they exist in micelles on the surface of the stream. The question now becomes whether chlorophenyl phosphoroamidates persist long enough in water to harm microorganisms in the environment.

CHAPTER FIVE

CHARACTERIZATION OF DICHLORO HYDROXY BENZENESULFONIC ACID

Introduction

The characterization of dichloro hydroxy benzenesulfonic acid detected in different effluent and natural water samples is carried out in three steps. First, we compared the product ion spectra of the benzenesulfonic acid from the environmental samples to spectra obtained from isomeric standards. Second, standard compounds having similar product ion spectra to the environmental samples were analyzed by cochromatography. Third, we evaluated similarity indices and contrast angle methods as a means of differentiating the structures of isomeric standard compounds and possibly the structures of different benzenesulfonic acids isolated from water taken from different sampling sites.

The structures of all sixteen dichloro hydroxybenzenesulfonic acids are shown below in Figure 29.

Four possible structures are 1,4-hydroxy benzenesulfonic acids (*para*-substituted), six possible structures are 1,3-hydroxy benzenesulfonic acids (*meta*-substituted), and another six possible structures are 1,2-hydroxy benzenesulfonic acids (*ortho*-substituted). Three of the four possible para substituted compounds were generated by sulfonation of dichlorophenol. Sulfonation of 3,5-dichlorophenol to prepare 2,6-dichloro-4-hydroxy benzenesulfonic acid (compound 4 in Figure 29) was unsuccessful due to the high ortho deactivating effect of chlorine atoms of 3,5-dichlorophenol. No meta isomer (compounds 5-10 in Figure 29) were generated

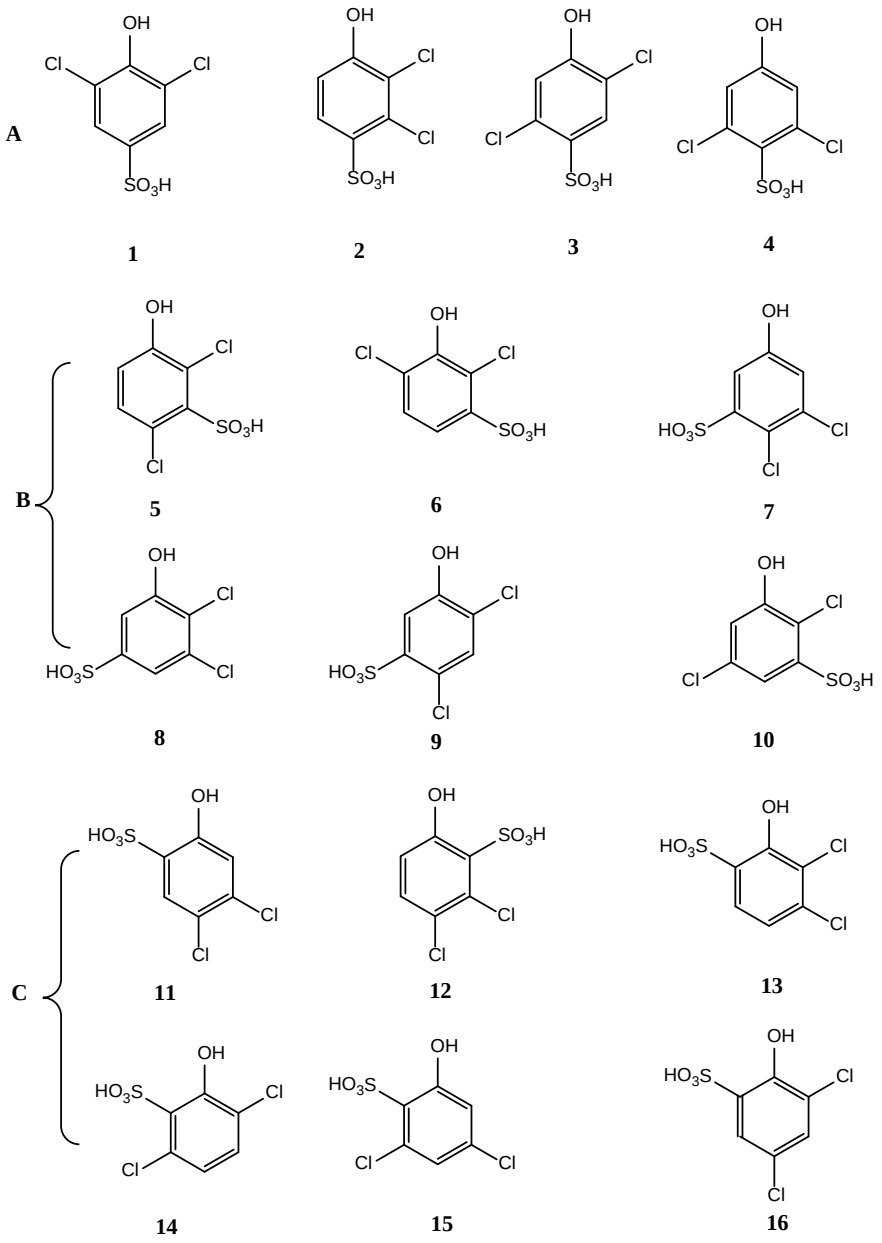


Figure 29. A: Structures of possible dichloro para-hydroxy benzenesulfonic acids (1) 3,5-dichloro- (2) 2,3-dichloro- (3) 2,5-dichloro and (4) 2,6-dichloro-4-hydroxy benzenesulfonic acid. B: Structures of possible dichloro meta-hydroxy benzenesulfonic acids (5) 2,6-dichloro- (6) 2,4-dichloro- (7) 5,6-dichloro- (8) 4,5-dichloro- (9) 4,6-dichloro- (10) 2,5-dichloro-3-hydroxy benzenesulfonic acid. C: Structures of possible dichloro ortho-hydroxy benzenesulfonic acids (11) 4,5-dichloro- (12) 5,6-dichloro- (13) 3,4-dichloro- (14) 3,6-dichloro- (15) 4,6-dichloro- (16) 3,5-dichloro-2-hydroxy benzenesulfonic acid.

(phenol groups are ortho, para directing). All of the six possible ortho substituted compounds were synthesized successfully.

Isomeric dichloro hydroxybenzenesulfonic acid standards were synthesized by Marlon Lutz in the laboratory of Dr. Daniel Becker at Loyola University. When all six of the isomeric dichlorophenols were sulfonated [54]**,** nine out of the sixteen potential isomers were formed, three *para* isomers and six *ortho* isomers were available for product ion analysis.

Product Ion Spectra of Standard Compounds

Product ion spectra of the synthetic standards are shown below in Figure 30 to Figure 38. The product ion spectra of para- isomers and ortho- isomers are distinctly different. The retention times of these isomers are also significantly different, para- isomers usually elute early (3-5 minutes) and ortho- isomers elute later (11 to 17 minutes). The major product ions of all of the nine standard compounds will be listed in Table 10 below.

Table 10. Major Product Ions of Dichloro Hydroxy Benzenesulfonic Acid Standard Compounds

Compound number	Isomers	Product Name	Product ions (m/z)
1	Para-	3,5-dichloro-4-hydroxy benzenesulfonic acid	35,80,113,177,205
2	Para-	2,3-dichloro-4-hydroxy benzenesulfonic acid	35,80,113,125,141,157,161,177,205
3	Para-	2,5-dichloro-4-hydroxy benzenesulfonic acid	35,80,113,141,157,177,205
11	Ortho-	4,5-dichloro-2-hydroxy benzenesulfonic acid	35,80,125,161
12	Ortho-	5,6-dichloro-2-hydroxy benzenesulfonic acid	35,80,125,161
13	Ortho-	3,4-dichloro-2-hydroxy benzenesulfonic acid	35,80,125,161
14	Ortho-	3,6-dichloro-2-hydroxy benzenesulfonic acid	35,80,125,161
15	Ortho-	4,6-dichloro-2-hydroxy benzenesulfonic acid	35,80,89,125,161
16	Ortho-	3,5-dichloro-2-hydroxy benzenesulfonic acid	35,80,125,161

The formation of these product ions will be listed in Table 11 below.

Table 11. Product Ions Observed in Analysis of Dichloro Hydroxybenzene Sulfonic Acids

Product Ion (m/z)	Formation
35	Cl^-
80	SO_3^-
89	Losses of SO_3 and 2HCl
113	Consecutive loss of HCl, SO_2, CO
125	Losses of SO_3 and HCl
141	Losses of SO_2 and HCl
157	Losses of SO and HCl
161	Loss of SO_3
177	Loss of SO_2
205	Loss of HCl

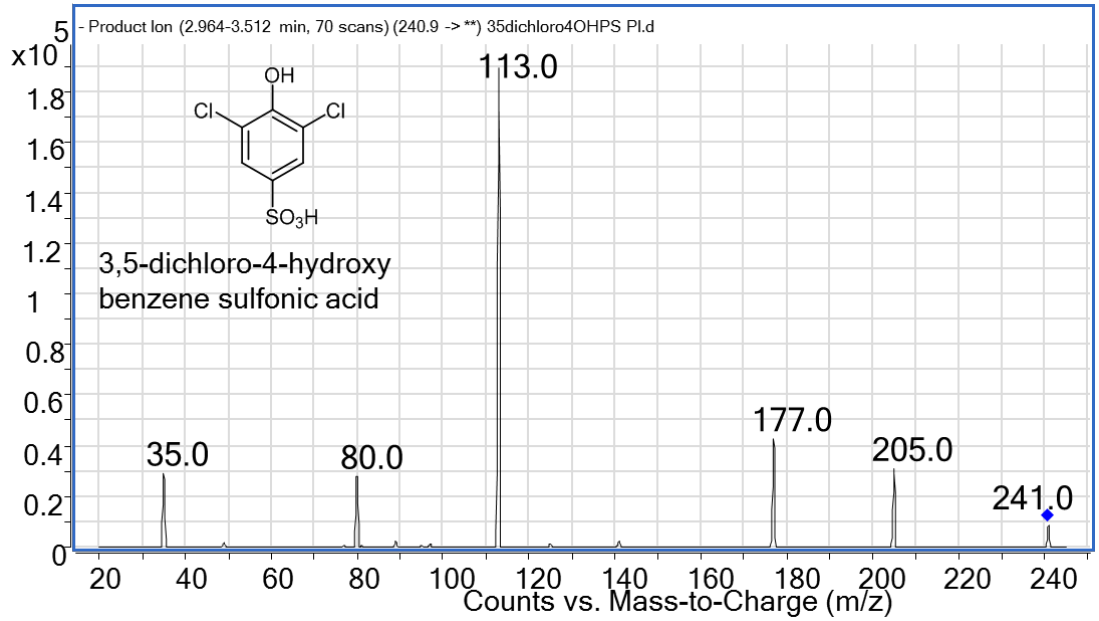


Figure 30. Product ion spectrum of compound 1 (3,5-dichloro-4-hydroxy benzenesulfonic acid)

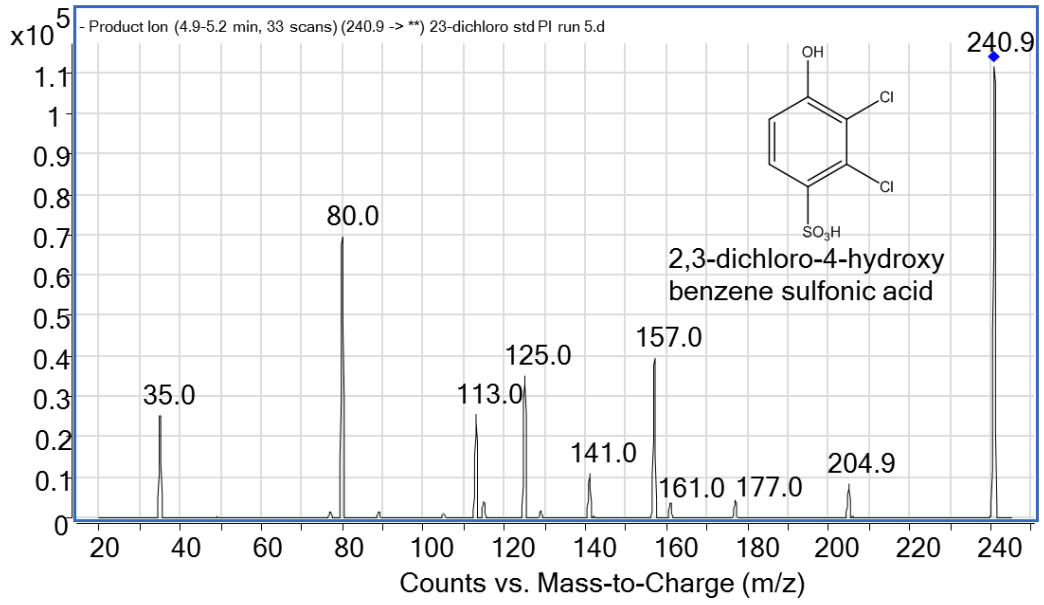


Figure 31. Product ion spectrum of compound 2 (2,3-dichloro-4-hydroxy benzenesulfonic acid)

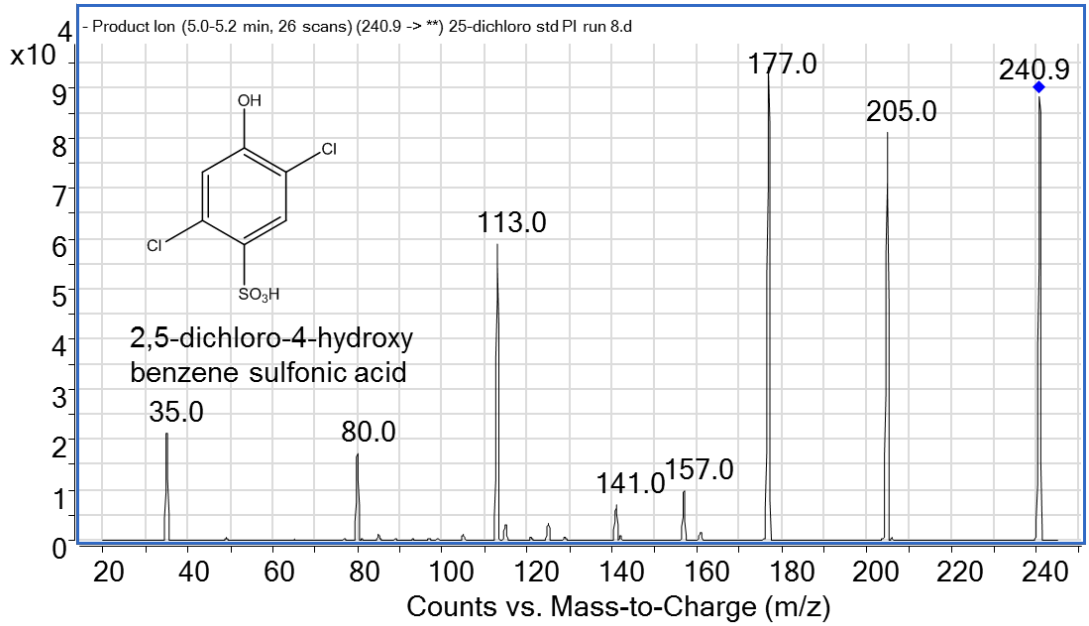


Figure 32. Product ion spectrum of compound 3 (2,5-dichloro-4-hydroxy benzenesulfonic acid)

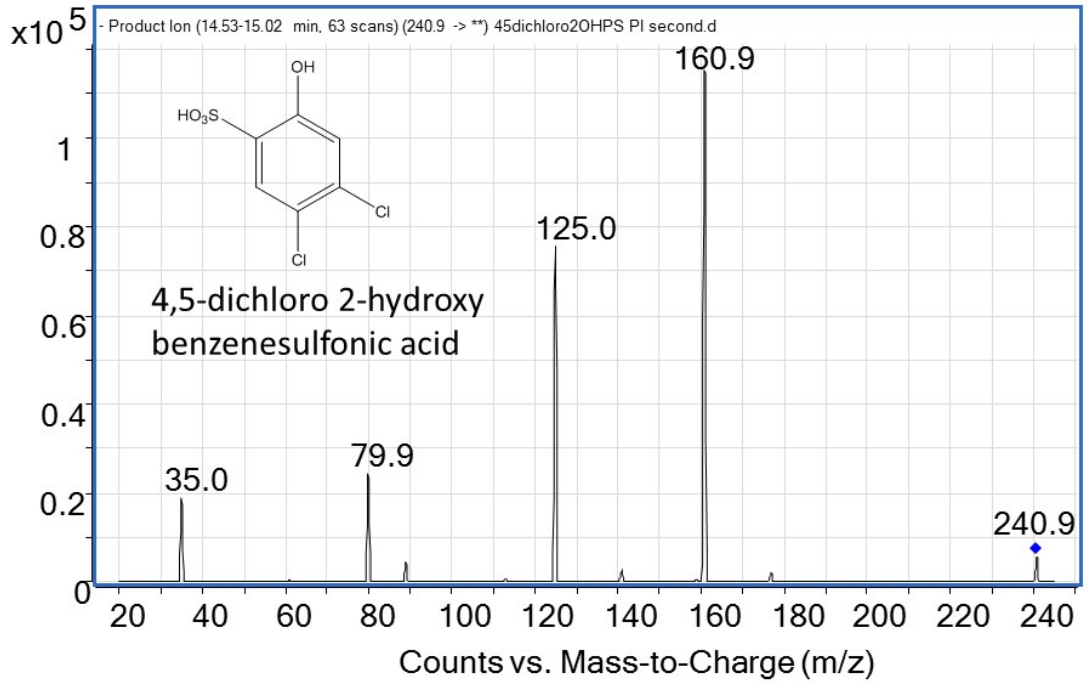


Figure 33. Product ion spectrum of compound 11 (4,5-dichloro-2-hydroxy benzenesulfonic acid)

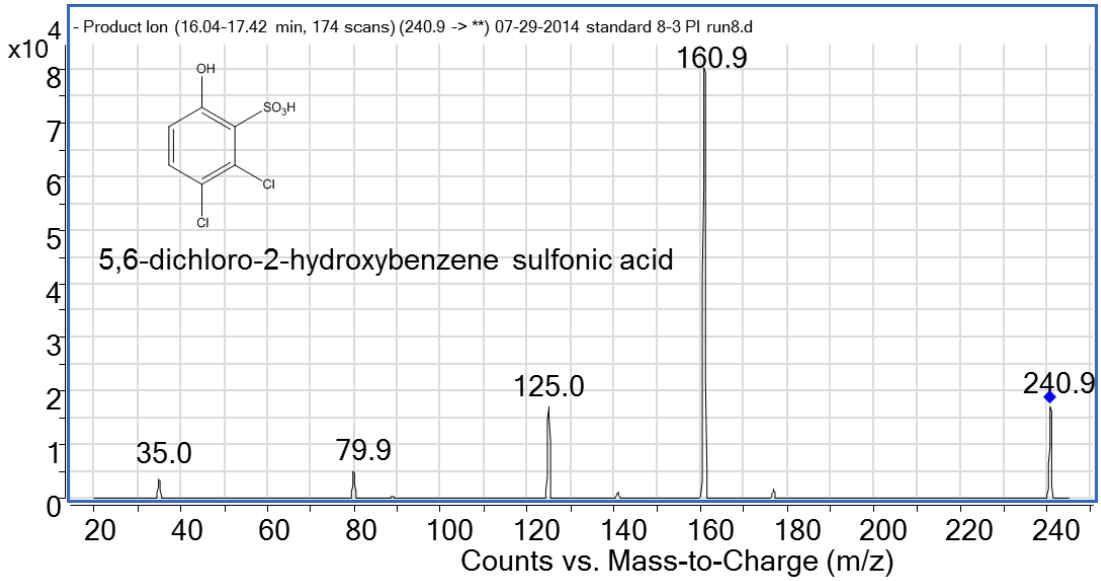


Figure 34. Product ion spectrum of compound 12 (5, 6-dichloro-2-hydroxy benzenesulfonic acid)

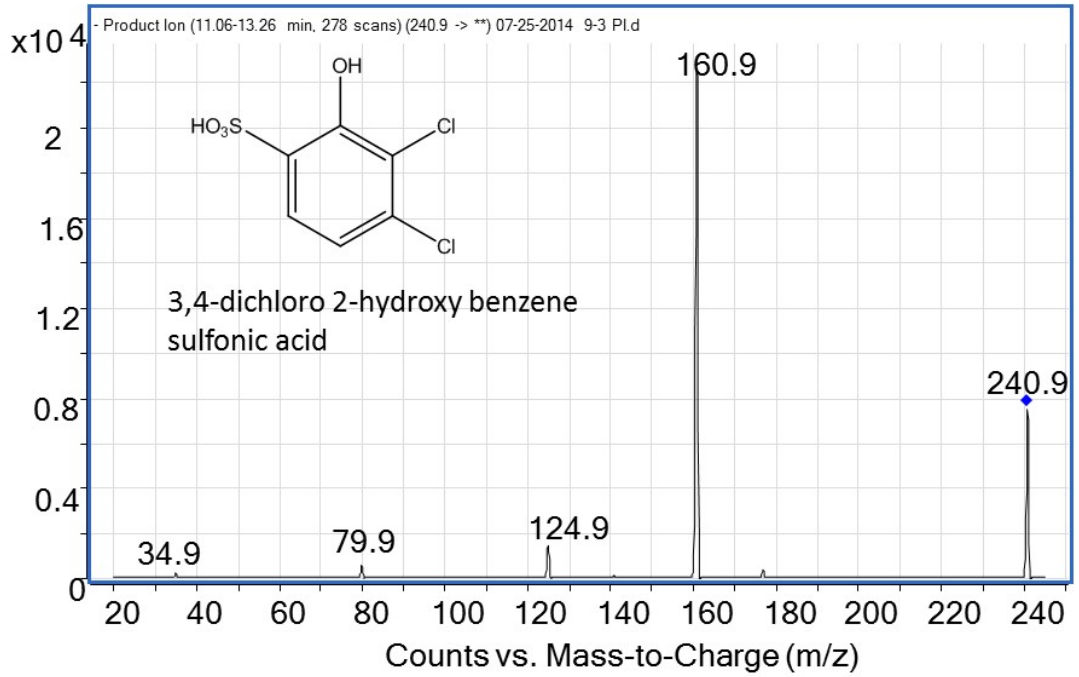


Figure 35. Product ion spectrum of compound 13 (3,4-dichloro-2-hydroxy benzenesulfonic acid)

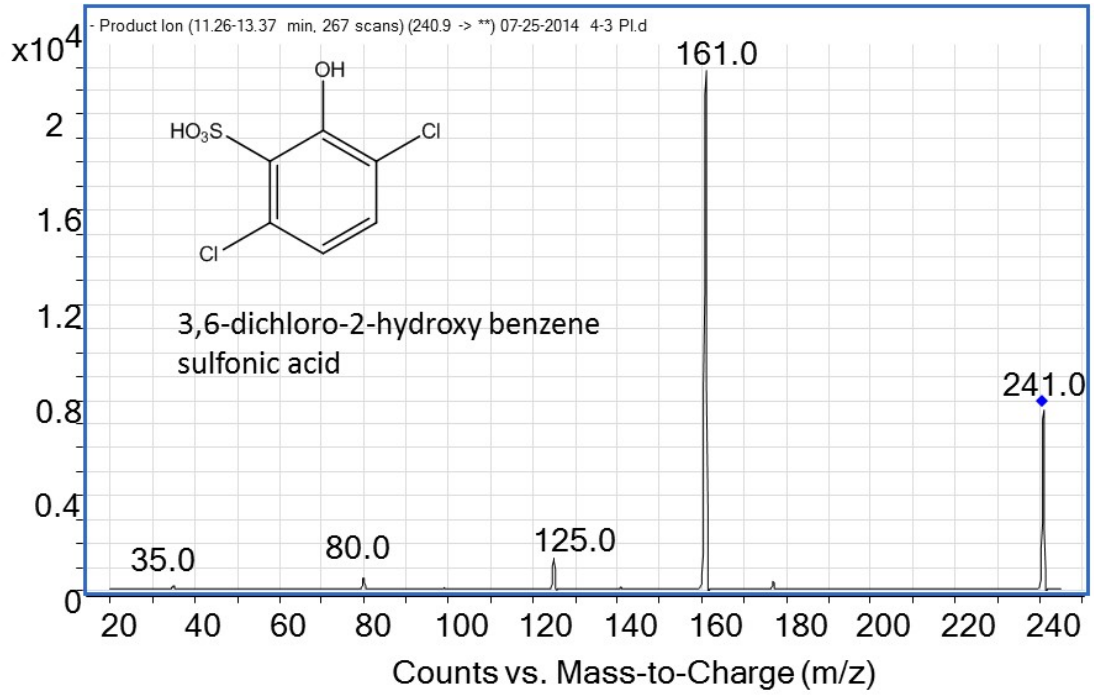


Figure 36. Product ion spectrum of compound 14 (3,6-dichloro-2-hydroxy benzenesulfonic acid)

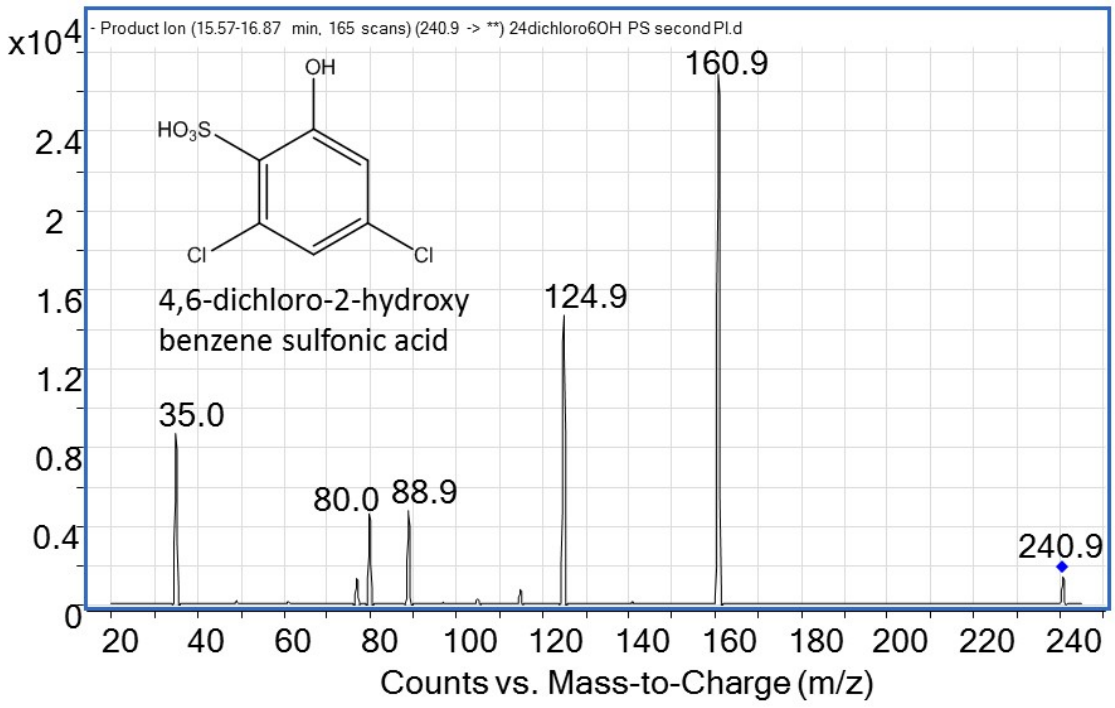


Figure 37. Product ion spectrum of compound 15 (4,6-dichloro-2-hydroxy benzenesulfonic acid)

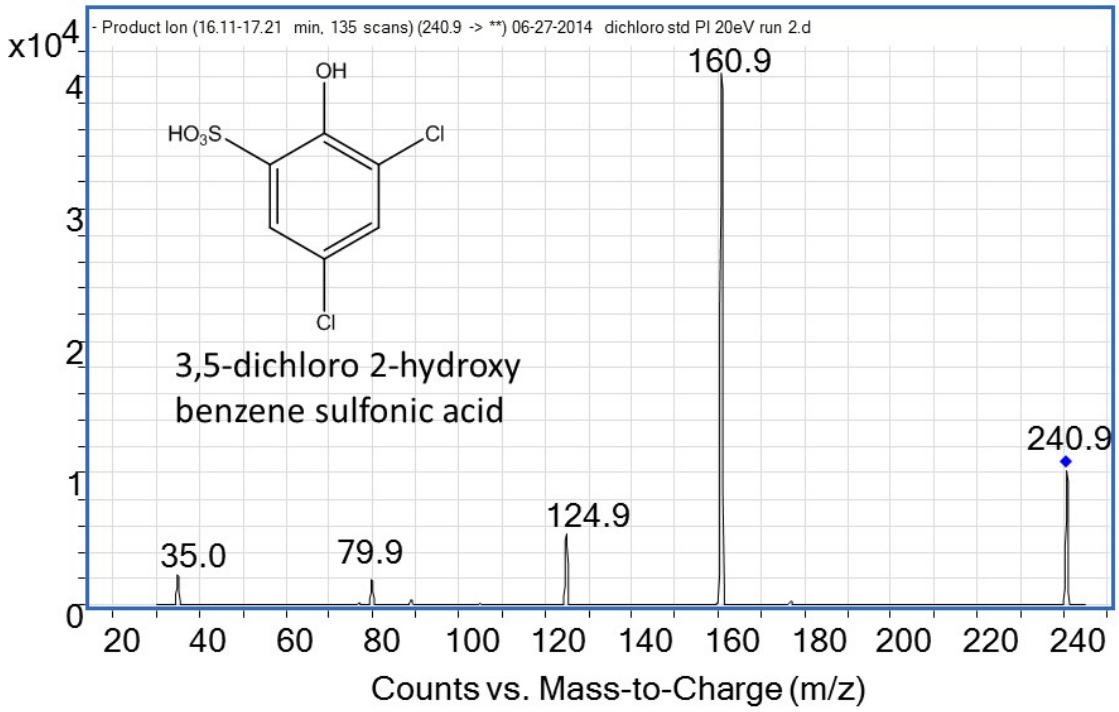


Figure 38. Product ion spectrum of compound 16 purchased from Sigma (3,5-dichloro-2-hydroxy benzenesulfonic acid)

Comparison of Standard Compounds with Unknown

Product ion spectra and retention times of the standard compounds are acquired with the same gradient used for discovery of unknown chlorinated pollutants. All of the six *ortho*- isomers of dichloro 2-hydroxy benzenesulfonic acids (compounds 11 to 16) eluted at much longer retention times (between 11.5 and 17.5 minutes) than the environmental samples (between 5 and 7 minutes), as well as the *para*- isomers of dichloro 4-hydroxy benzenesulfonic acid (compounds 1 to 3) when analyzed with the 30 minute water/methanol gradient. Also, the product ion spectra of the *ortho*-substituted isomers (Figure 33 to 38) are distinctly different than the product ion spectra of the m/z 240.9 derived from the natural water samples. The product ion spectra of the *ortho*-substituted hydroxy benzene sulfonic acids are dominated by an ion at m/z 161, which is formed by the inductive cleavage of the SO_3 from the benzene ring, and none of the *para*-substituted isomers (Figure 30 to 32) and natural water samples' product ion spectrum shows m/z 161.

Neither the product ion spectra of the m/z 240.9 ions suggested to be dichloro hydroxy benzenesulfonic acids in the extracted water samples (Figure 22 Stickney waste water effluent and Daley Park) nor the product ion spectra of the *para*-substituted hydroxy benzenesulfonic acids has m/z 161. Therefore, none of the sulfonic acids detected in natural water or treatment plant effluent are suggested to be *ortho*-substituted hydroxy benzenesulfonic acids. The m/z values of the product ions observed in Figure 30 (m/z 113, m/z 177, m/z 205, m/z 80, m/z 35) are consistent with the product ion spectra of the m/z 241 ion isolated from waste water effluent and Daley Park sample along Chicago River SSC shown in Figure 22. Therefore, the dichloro hydroxy- benzenesulfonic acids detected in waste water effluent and Daley Park (Chicago River) sample are suggested to be one of the *para*-substituted isomers.

As the possibility of unknown being the *ortho*- substituted isomer has been precluded, in order to confirm which *para*- isomer is the unknown discovered in waste water effluent and natural water samples, experiments were carried out to compare the retention times of the unknown dichlorinated species with those of the three *para* dichloro hydroxy benzenesulfonic acids formed by the sulfonation of the dichlorophenols. Cochromatography was carried under isochratic conditions using a 5% methanol in water mobile phase to lengthen the retention times of the isomeric standards and those of the dichlorinated m/z 241 ion isolated from different water sources. Chromatograms showing the retention times of the m/z 241 ions isolated from four different water samples are shown in Figure 39.

Figure 39 shows the chromatograms of dichlorinated species m/z 241 extracted from water samples obtained from Stickney waste water effluent (Figure 39A), Cicero Ave near Sanitary Ship Canal (SSC) along Chicago River (Figure 39B), Erie Street at SSC (Figure 39C) and Daley Boat Launch at SSC (Figure 39D). The similarities in the retention times for the m/z 241 ion isolated from the different water samples suggest they have the same *para*-hydroxy-substituted structure.

In order to identify which para- isomer has the same molecular structure as the unknown(s), the retention times of the three isomers were compared under the same isochratic conditions. Their chromatograms are shown in Figure 40. The chromatograms in Figures 39 and 40 suggest that the dichlorinated hydroxy benzenesulfonic acids isolated from water samples taken from different places around the Chicago metropolitan area all have structures consistent with 3,5-dichloro-4-hydroxy benzenesulfonic acid (compound 1 in Figure 29) with a retention time of ca. 14.4 minutes. The other two standards, 2,5-dichloro- (compound 3 in Figure 29) and 2,3-dichloro- (compound 2 in Figure 29) 4-hydroxy benzenesulfonic acid eluted with retention

times of 11.3 and 11.6 minutes under these conditions. Therefore, we conclude that the dichloro hydroxy benzene sulfonic acid detected in the effluent wastewater and natural water samples is 3,5-dichloro-4-hydroxy benzenesulfonic acid (Figure 40C).

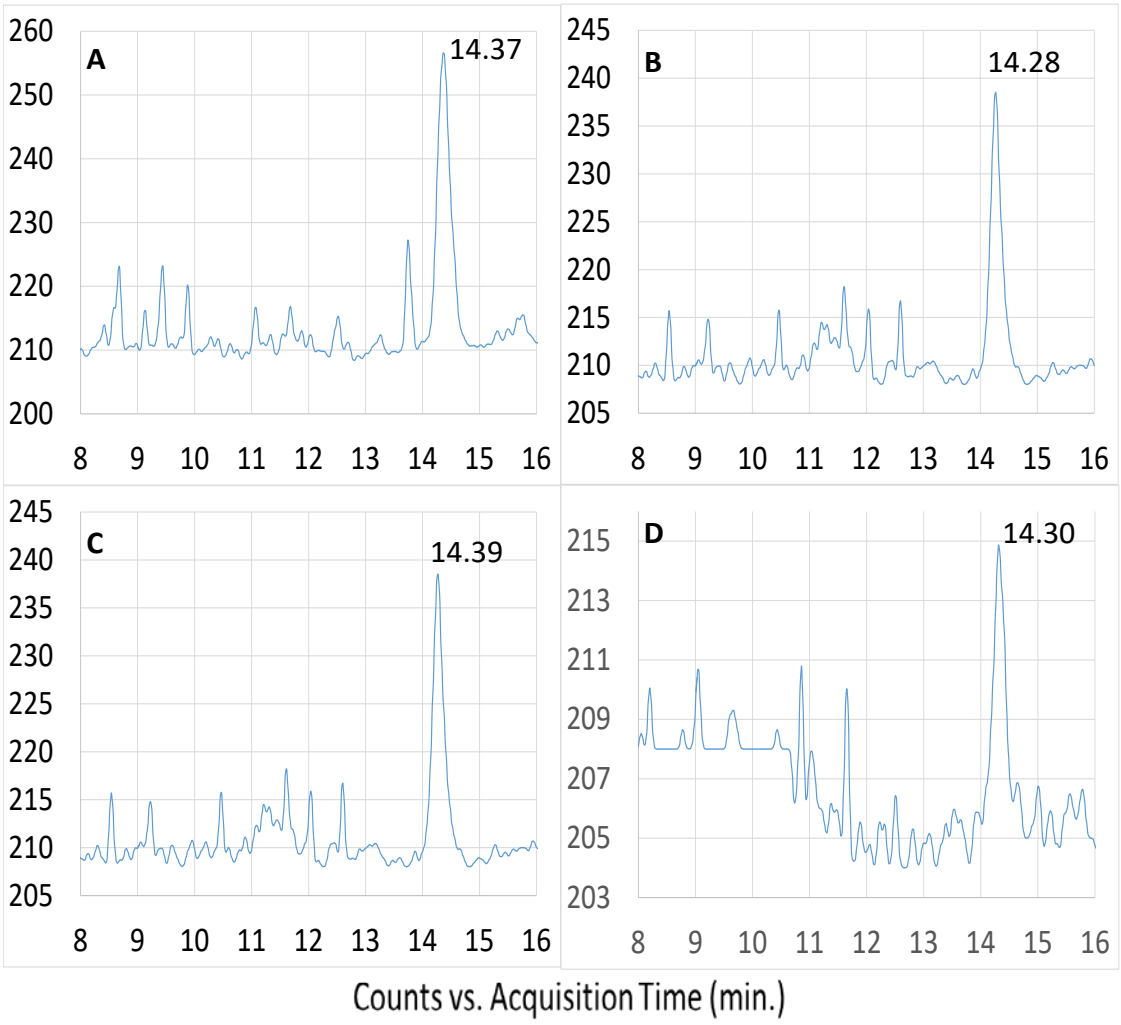


Figure 39. Chromatograms of dichlorinated m/z 241 ion isolated from water samples taken from (A) Stickney wastewater effluent, (B) Cicero SSC, (C) Erie SSC, and (D) Daley SSC. The standard deviation associated with the retention times (N = 3) is ± 0.06 minutes

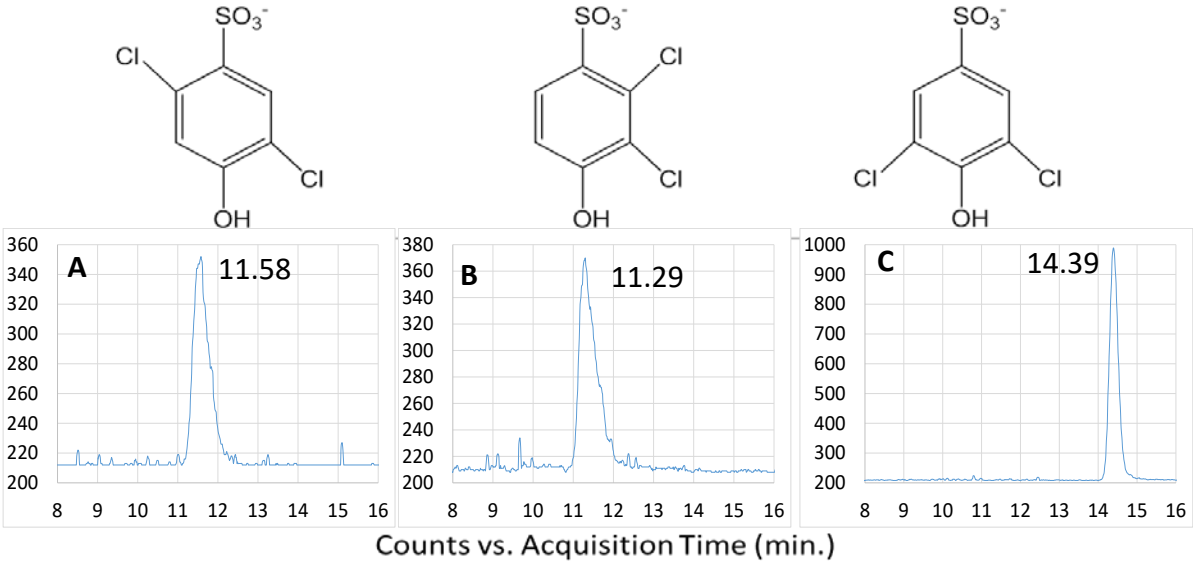


Figure 40. Chromatograms of (A) 2,5-dichloro- (B) 2,3-dichloro- and (C) 3,5-dichloro-4-hydroxy benzenesulfonic acid

Dichloro 1,3-Hydroxybenzene Sulfonic Acid Isomers: Other Considerations

The six *meta*- isomers of dichloro hydroxy benzenesulfonic acids (Figure 29 B) and 2,6-dichloro-4-hydroxy benzene sulfonic acids (Figure 29 A compound 4) were not included in this study as standard compounds because there do not appear to be any published synthetic methods or commercial sources for any of these compounds to date. 1,3-Hydroxybenzene sulfonic acid as a starting material for the production of the dichloro 1,3-hydroxybenzene sulfonic acids (by direct chlorination) is relatively expensive (ca. $2,950/gram) [55], therefore it is not likely that large quantities of this material would be released into the environment on a large scale as a manufacturing by-product. Although it cannot be stated with absolute certainty that they are not present in any of the water samples analyzed, however, the difficulty and expense of generating the *meta* isomers would suggest that they are not present in the environment as pollutants. The synthesis of these *meta* substituted isomers would require more individual synthetic steps than the preparation of the *ortho* and *para* hydroxy benzenesulfonic acids, given that phenols are

ortho and *para* directing, again significantly decreasing the likelihood that they would be used industrially on such a scale to be detected in the environment. The production of the *meta*-substituted isomers would be precluded in wastewater treatment, in consideration of well-known patterns of electrophilic aromatic substitution. 1,3-Hydroxy-benzenesulfonic acid as a starting material for the production of the dichloro 1,3-hydroxy-benzenesulfonic acids is relatively expensive. Therefore it is not likely that large quantities of this material would be released into the environment on a large scale as manufacturing by-product.

The generation of 2,6-dichloro-4-hydroxy benzenesulfonic acid as a synthetic product with any starting material is unlikely because it requires two chlorine atoms be adjacent to the sulfonic acid group on the benzene ring, which is precluded by both steric and electronic considerations.

Applications of Dichloro Hydroxy Benzenesulfonic Acid

There is evidence to suggest that 3,5-dichloro-4-hydroxy benzenesulfonic acid is a by-product of the dye industry [50]. This compound may be attached to a dye molecule or some other intermediate as a phenol ester, being one of the components of a “labeling reagent” (Figure 41). Labeling reagents are composed of dye molecule (-R in Figure 41) connected to 3,5-dichloro-4-hydroxy benzenesulfonic acid by covalent bonds. The sulfonic acid group of 3,5-dichloro-4-hydroxy benzenesulfonic acid enhances the solubility of the dye in water, so that the dye molecule could be applied in hydrophilic environment. The dye molecule or other intermediate is usually called “reporter molecule” (-R in Figure 41), that is detected/recognized by a characteristic absorbance.

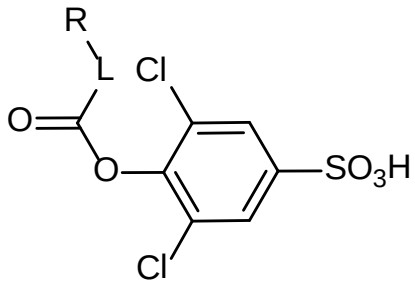


Figure 41. Application of 3,5-dichloro-4-hydroxy benzenesulfonic acid as labeling reagent

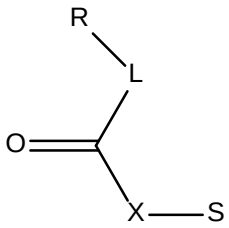


Figure 42. Labeling reagent bonded to a substrate

In Figure 41 and 42, L is a linker group, which could be O, S, CH_2 etc. A general structure of the labeling reagent attached to substrate is shown in Figure 42. In Figure 42, R is the dye or reporter molecule, L is the linker, X is a nucleophile group and S is the substrate molecule.

Another application of 3,5-dichloro-4-hydroxy benzenesulfonic acid as a labeling reagent was described in a patent [51]. The structure of labeling reagent is the same as shown above in Figure 41. The application is to activate a molecule that contains alkyne group, and then facilitate the cyclo-addition reactions such as Diels-Alder reaction. Substrates used in this patent are mostly biomolecules.

Other than the by-product of the dye industry, 3,5-dichlorophenol-4-hydroxy-benzenesulfonic acid may be formed during the water treatment process. 4- Hydroxy benzenesulfonic acid is a by-product of the electroplating industry [52]. The 3,5- dichloro-4-hydroxy benzenesulfonic acid would be the favored dichlorination product based on the

activating/deactivating directing properties of the hydroxyl and sulfonic acid groups attached to the benzene ring. If chlorination of the 4-hydroxy benzene sulfonic acid were a significant source of 3,5-dichloro-4-hydroxy-benzenesulfonic acid, one might expect to find the monochloro product as well. It has not been observed any ion suggested to be a monochlorophenol sulfonic acid in any single reaction monitoring analysis of extracted natural or effluent wastewater samples to date. Although 4-hydroxy benzenesulfonic acid is not the precursor for 3,5-dichlorophenol-4-hydroxy-benzene sulfonic acid in this study, it does serve as the precursor of a series of iodo-disinfection byproducts in chlorinated saline waste water effluents in Hong Kong [53].

CHAPTER SIX

EVALUATION OF THE USE OF SIMILARITY INDICES BASED ON PRODUCT ION SPECTRA FOR THE DIFFERENTIATION OF ISOMER STRUCTURES

Introduction

Retention times in some instances may not be sufficiently different to differentiate the structures of two isomers. In the case when isomeric standards of known structure are not available, the comparison of product ion spectra of two unknowns may be carried out so as to ask the question "Are the structures of these two pollutants the same or different?" This is especially important when compounds having the same empirical formulas are detected in water samples acquired from two different sites at two different times.

There are two parameters, similarity index (SI) and spectral contrast angle (θ), are used to distinguish product ion spectra. Spectral contrast angle method is superior to the similarity index, in cases where very similar spectra can be distinguished [42]. Spectral contrast angle has the advantage to differentiate spectra in which most of the peaks are similar and only small differences exist, while similarity index method claims they are same. Similarity index involves the sum of differences of relative abundances of the chosen product ions in two respective spectra and can be viewed as an average standard deviation [42], whereas the spectral contrast angle, measuring the angle between the two vectors to represent the two spectra to be compared, involves the sum of the product of two relative abundances of chosen peaks. Spectral contrast angle, a special case of the dot-product algorithm, is a better parameter used for spectra search,

returning 75% of the correct match at the top of the hit list [45]. SI and spectra contrast may be useful to distinguish the structures of isomers based on relative abundance when the fragmentation pathways are the same. Similarity index and spectral contrast angle are used to the product ion spectra of ions suggested to be dichloro hydroxy benzenesulfonic acids isolated from water sampled at different locations (Chicago River and Stickney WWTP) and standard compounds for the purpose of distinguishing isomers. The product ion spectra dichloro hydroxy benzenesulfonic acid standards will be compared as well.

Similarity Index and Spectral Contrast Angle

Similarity index (SI) and spectral contrast angle (θ) are two commonly used parameters to compare mass spectra [44]. Equations describing the calculation of similarity indices and spectral contrast angles are shown below in equation 6-1 and 6-2, respectively [42, 46].

$$SI = \sqrt{\frac{\sum \left[\frac{(i - i_0)}{(i + i_0)} \times 100\right]^2}{N}} \quad (6\text{-}1)$$

Here i and i_0 are the relative abundances of two ions of the same m/z value in the corresponding two product ion spectra and N is the total number of ions used for calculation in the product ion spectrum.

The second parameter used to compare isomer product ion spectra is spectral contrast angle:

$$\cos\theta = \frac{\sum_i a_i b_i}{\sqrt{\sum_i a_i{}^2 \sum_i b_i{}^2}} \quad (6\text{-}2)$$

Here a_i and b_i are the relative abundances of the same m/z in the two respective product ion spectra, A and B. A schematic diagram indicating the definition of spectral contrast angle is

shown in Figure 43.

In Figure 43, only three ions per spectrum are shown in the scheme for simplicity, although as many as ions in the product ion spectra can be used in the calculations of spectral contrast angle. An N-dimensional vector is constructed when different m/z are used. The lengths of the vectors A and B (r_a and r_b) are determined by equation 6-3 and 6-4.

$$r_a = \sqrt{\sum_i {a_i}^2} \quad (6\text{-}3)$$

$$r_b = \sqrt{\sum_i {b_i}^2} \quad (6\text{-}4)$$

Since the dot product of vector A and vector B is defined as:

$$A \cdot B = r_a\, r_b \cos\theta \quad (6\text{-}5)$$

$$A \cdot B = \sum_{i=1}^{n} aibi \quad (6\text{-}6)$$

Thus, by solving cos θ from equation 6-5 and 6-6, equation 6-2 can be obtained. From the definition of cos θ, an angle of zero means that the two spectra are identical and an angle of 90° indicates a maximum spectral difference.

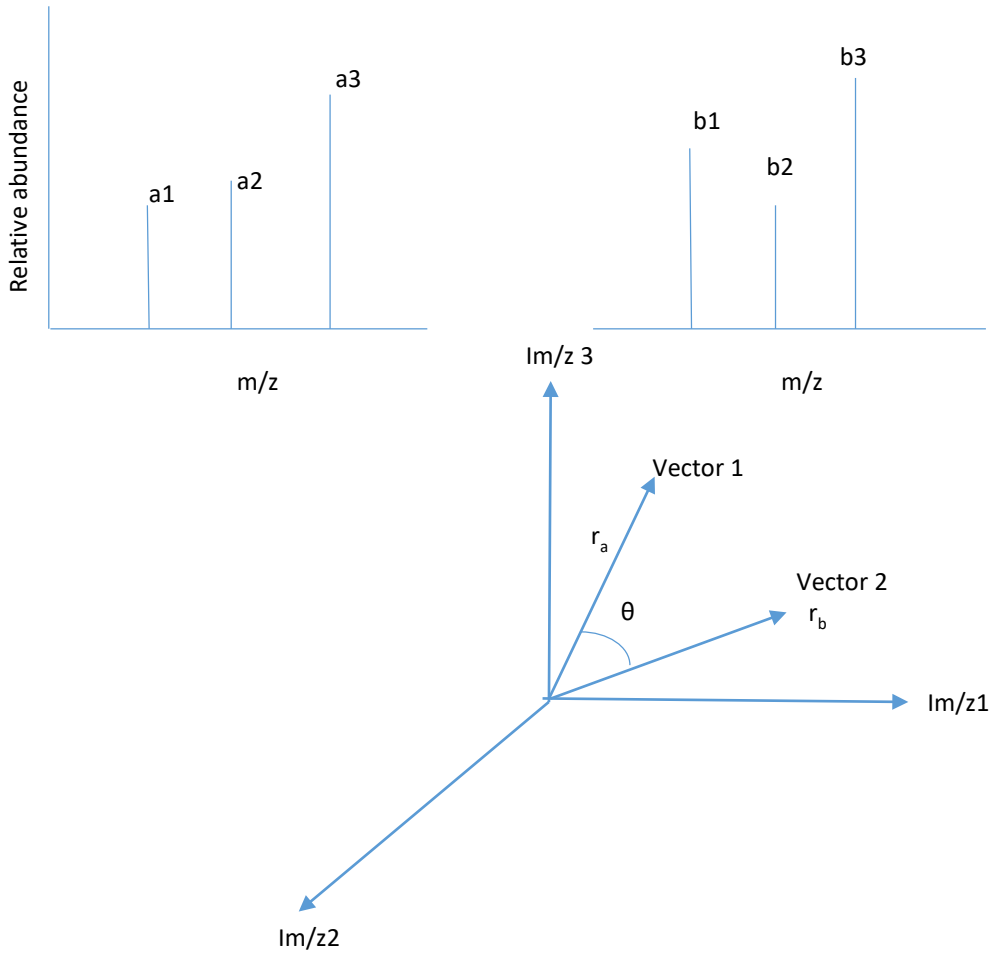


Figure 43. Schematic diagram of spectral contrast angle

Evaluation of Product Ion Spectra of Standard Compounds Using Similarity Indices

We will compare the product ion spectra of three para-substituted (-OH vs. -SO_3H) and six ortho-substituted (-OH vs. -SO_3H) dichloro hydroxybenzene sulfonic acids. Product ion spectra of three para-standards, 3,5-dichloro-4-hydroxy benzenesulfonic acid (compound 1) and 2,3-dichloro-4-hydroxy benzenesulfonic acid (compound 2) and 2,5-dichloro-4-hydroxy

benzenesulfonic acid (compound 3) were obtained. The product ion spectra of the 1,2 and 1,4 hydroxybenzene sulfonic acids are sufficiently different (unique fragmentation pathways) that they can be differentiated without similarity index or contrast angle calculations. Similarity indices and spectral contrast angles calculated between any para isomer and ortho isomer pair would be large due to their distinctively different product ions.

Self Similarity Index (Self-SI) and Self Spectral Contrast Angle (Self-θ) of Three Standard Compounds

Self similarity index (Self-SI) and self spectral contrast angle (Self-θ) are calculated as the averages of all the combinations of pairwise comparisons of the product ion spectra set obtained for the same compound. The SI for each pair is calculated according to equation 6-1 and θ for each pair is calculated according to equation 6-2. Therefore, for a set of product ion spectra of 12, all possible comparisons equal $^{12}C_2 = 12!/(2!\times(12-2)!) = 66$ and self-SI is the average of 66 SI values for this example. Then we compare the spectra of one isomer to another in the same way and calculate an inter SI or theta. If the two self-comparisons are different from the comparison of different isomers, then we can say the two compounds being compared have a different structure.

The average relative abundances of the product ions observed in the spectra of the 1,4 isomers collected at 13eV collision energy are shown below in Table 12.

The self-SI and self-θ associated with individual isomers are given below in Table 13.

The values of self-SI and self-θ serve as a criteria to distinguish product ion spectra, so the inter-SI and inter-θ must be significantly different than the values of self-SI and self-θ. Other than collision energy of 13eV, collision energy of 20eV is also utilized for the product ion study

of these three standards. Table 14 shows the relative abundances of product ions in the product ion spectra obtained at 20eV.

Table 12. Relative Abundances of Product Ions Calculated from Product Ion Spectra Obtained at 13eV of Three Para-Substituted Standard Compounds

Standard Compound	3,5-dichloro 4-hydroxy benzene sulfonic acid **(1)**	2,3-dichloro 4-hydroxy benzene sulfonic acid **(2)**	2,5-dichloro 4-hydroxy benzene sulfonic acid **(3)**
m/z 35	5.61±0.23	38.47±1.31	13.51±0.44
m/z 80	5.19±0.18	100±0.00	11.64±0.27
m/z 113	51.89±2.18	41.24±1.28	36.77±0.89
m/z 125	----------	49.25±1.99	1.55±0.16
m/z 141	----------	28.71±1.32	9.04±0.34
m/z 157	----------	91.33±2.27	11.08±0.36
m/z 161	----------	7.80±0.58	1.70±0.23
m/z 177	44.30±1.83	6.89±0.60	67.39±0.66
m/z 205	100±0.00	18.86±0.50	100±0.00

Table 13. Self-SI and Self-θ of Standard Compounds Calculated from Product Ion Spectra Obtained at 13eV

Compound number	Self Similarity Index	Self Spectral Contrast Angle
1	2.01±1.32	0.02±0.01
2	3.14±1.74	0.02±0.01
3	3.54±2.03	0.01±0.005

Table 14. Relative Abundances of Product Ions Calculated from Product Ion Spectra Obtained at 20eV of Three Para-Substituted Standard Compounds

Standard Compound	3,5-dichloro 4-hydroxy benzene sulfonic acid	2,3-dichloro 4-hydroxy benzene sulfonic acid	2,5-dichloro 4-hydroxy benzene sulfonic acid
m/z 35	8.65±0.27	36.49±0.84	22.35±0.40
m/z 80	8.30±0.10	100±0.00	18.44±0.43
m/z 113	100±0.00	36.12±0.73	61.76±1.35
m/z 125	----------	52.26±0.98	3.31±0.23
m/z 141	----------	15.36±0.53	7.68±0.24
m/z 157	----------	56.27±1.23	10.20±0.23
m/z 161	----------	5.36±0.13	1.75±0.14
m/z 177	68.37±0.81	6.50±0.23	100±0.00
m/z 205	92.93±2.22	11.67±0.37	85.26±1.28

From Table 12 and Table 14, the relative abundances of these product ions changed with the increase of collision energy. In Table 12, where the product ion spectra were obtained at

13eV, m/z 205 is the most abundant ion in the product ion spectra of 3,5-dichloro-4-hydroxy benzenesulfonic acid and 2,5-dichloro-4-hydroxy benzenesulfonic acid, whereas m/z 80 is the most abundant ion in the product ion spectra of 2,3-dichloro-4-hydroxy benzenesulfonic acid. In Table 14, where the product ion spectra were obtained at 20eV, m/z 80 is still the most abundant product ion of 2,3-dichloro-4-hydroxy benzenesulfonic acid, but for 3,5-dichloro 4-hydroxy benzenesulfonic acid; however, the most abundant ion becomes m/z 113 and for 2,5-dichloro 4-hydroxy benzenesulfonic acid, the most abundant ion is m/z 177. In this study, the relative abundances are normalized to the most abundant product ion in the relative product ion spectrum.

We increased the collision energy to 20 eV to see how this would change the self-SI and theta calculations. The result of self-similarity index and self-spectra contrast angle of each standard compound calculated from the comparison of the set of product ion spectra acquired at the collision energy of 20 eV to itself is shown in Table 15.

Table 15. Self-SI and Self-θ of Standard Compounds Calculated from Product Ion Spectra Obtained at 20eV

Compound number	Self SI	Self θ
1	1.08±0.67	0.015±0.010
2	1.54±0.68	0.016±0.008
3	2.29±1.20	0.012±0.007

The data in tables 13 and 15 suggests that product ion spectra collected with 20 eV collision energy are more reproducible than product ion spectra acquired with 13 eV energy because the self-SI values are much closer to one, suggesting that the relative abundances being measured in each product ion spectrum compared are more reproducible than those measured in the 13 eV spectra.

In the next section, the inter similarity indices and inter spectral contrast angles calculated from the comparison between the product ion spectra sets of three standard compounds obtained from both collision energy, 13eV and 20eV will be discussed.

Inter Similarity Index (Inter-SI) and Inter Spectral Contrast Angle (Inter-θ) of Three Standard Compounds

Inter similarity index (inter-SI) and inter spectral contrast angle (inter-θ) are calculated as the averages of all the combinations of pairwise comparisons of the product ion spectra set obtained for the two different compounds. SI for each pair is calculated according to equation 6-1 and θ for each pair is calculated according to equation 6-2. Therefore, for a set of product ion spectra of 8, all combination of comparison equals 8×8 = 64. The inter-SI and inter-θ obtained from the comparison of product ion spectra of three standard compounds acquired at the collision energy of 13eV are shown below in Table 16.

Table 16. Inter-SI and Inter-θ of Three Para Substituted Standard Compounds Calculated from Product Ion Spectra Obtained at 13eV

Compound Pairs	Inter SI	Inter θ
1 vs. 2	83.33±0.13	1.305±0.004
2 vs. 3	80.31±0.24	1.224±0.004
3 vs. 1	69.84±0.20	0.259±0.006

It can be seen from Table 16 that these three para standard compounds are different from each other, since their inter-SI is 83 for compound 1 vs 2, 80 for compound 2 vs 3 and 69 for compound 1 vs 3. All of the three inter similarity indices are significantly larger than the self-SIs of the three standard compounds (Table 13 and 15). The inter θ are significantly larger than the self θ as well. For example, the inter-θ for compound 1 vs. 2 is 1.305, if we convert this number from radian to degree, it will be 1.305 × 57.3 = 74.8 degree. The self-θ for compound 1 is 0.015

and equals to 0.015×57.3 = 0.86 degree. Therefore, inter-SI and inter-θ could be applied to distinguish product ion spectra of the three standard compounds.

The inter SI and inter θ of the three standard compounds calculated from the product ion spectra obtained at collision energy of 20eV is shown below in Table 17.

Table 17. Inter-SI and Inter-θ of Three Para Standard Compounds Calculated from Product Ion Spectra Obtained at 20eV

Compound Pairs	Inter SI	Inter θ
1 vs. 2	85.66±0.11	1.268±0.004
2 vs. 3	63.03±0.34	1.201±0.004
3 vs. 1	70.18±0.12	0.363±0.008

Rather large differences are apparent when self-SIs and thetas (Table 13 and 15) are compared to the inter-SIs and thetas in Table 16 and 17. The self-SIs range from 1 to 3.5, and self-θs range from 0.01 to 0.02, while inter-SIs and inter-θs in Table 16 and 17 are at the very least a factor of twenty larger than the self-comparisons (69.84 to 3.54, inter SI of compound 3 vs. 1 to self SI of compound 3). For example, compounds 1 and 2 have self-SIs of 1.08 (±0.67) and 1.54 (±0.68) while their inter SI at 20eV is 85.66 (±0.11), which is 79.31 and 55.62 times larger than the respective self SIs. Therefore, we would conclude that compound 1 and 2 have different structures because the self-SIs associated with these two compounds are so different from the SI obtained when product ion spectra from different compound are compared.

So far, all product ion spectra compared were acquired at the same collision energy. Because the relative abundances of product ions change with the increasing collision energy, we would like to determine whether the values of inter-SI and inter-θ calculated from the comparison of two sets of eight product ion spectra obtained at different collision energy for the same standard compound are significantly different.

For these comparisons, a set of eight product ion spectra obtained at 13eV are compared to the eight product ion spectra obtained at 20eV for the same standard compound. The inter SI and inter θ of the three para-standards are listed in Table 18. Since the relative abundances of product ions of all of the three standards changed with the increase of the collision energy, relatively larger inter-SI and inter-θ are expected.

Table 18. Inter-SI and Inter-θ of Three Standards Calculated from Product Ion Spectra Obtained at Collision Energy 13eV vs. 20eV.

Compound number	Inter-SI	Inter-θ
1 vs. 1	22.20±1.42	0.33±0.02
2 vs. 2	16.83±1.68	0.22±0.01
3 vs. 3	25.27±1.20	0.29±0.01

The data in Table 18 indicate that significant differences in product ion spectra are observed when the collision energy is increased from 13 eV to 20 eV. As an example the self-SIs for compound 3 at 13 eV and 20 eV are 3.54±2.03 and 2.29±1.20, respectively (Tables 13 and 15). When the 13 eV and 20 eV collision energy are used to calculate an Inter-SI, a value of 25.27 ±1.20 is obtained. The Inter-SI is at least a factor of seven larger than the largest self-SI. These results suggest that the product ion spectra of these dichloro hydroxy benzene sulfonic acids can change significantly as a function of collision energy.

Evaluation of Product Ion Spectra of Unknown using Similarity Indices

Here we evaluate the utility of similarity indices and spectra contrast angle analyses as a means of assessing whether or not compounds isolated from different environmental water samples at different times have the same structure. As mentioned in Chapter 5, the compound 3,5-dichloro-4-hydroxy benzenesulfonic acid (compound 1, above) was identified as a persistent pollutant through the comparison of chromatographic retention times and product ion spectra of standard compounds. Product ion spectra of the m/z 241 ion isolated from water samples taken from three

different sites at two different times compared using spectra contrast angle and similarity indices are evaluated to determine whether or not a definitive statement may be made regarding the identities of the compound(s) isolated from different water samples.

We have compared product ion spectra of the m/z 241 ion derived from water samples acquired at three different locations along the Chicago River (Daley, Erie, and Cicero) at two different times (2014 and 2015) each. Self-similarity indices and self-contrast angles were calculated for each of the twelve or eight sample groupings (as described above, Tables 19 and 20). Inter-SIs and contrast angles were calculated for each pairs of sites sampled within the same year (within several days of each other, Tables 21 and 22), and then between the same sites sampled at different times (Table 23).

When the data in Tables 19 to 23 is compared to the isomer differentiation studies carried out at 20 eV collision energy summarized in Tables 15 and 17, two observations stand out. The self-SIs and self-thetas are a factor of three or four larger in the comparison of spectra acquired from the same environmental sample than those in the comparison of spectra acquired from the same standard isomer. Secondly, the cross comparisons of product ion spectra acquired from different sites at different times are much smaller (again, a factor of three or four) than the cross comparisons of different isomer product ion spectra described in Table 17.

The SIs and thetas cross comparisons of the different samples taken from different sites and times appear to be equivalent to the Inter-SIs and thetas of the same standard compounds taken at different collision energies (Table 18).

We do know from the cochromatography studies described in chapter 5 that the identities of compounds forming the m/z 241 ion from all sites taken at different times (3,5-dichloro-4-

hydroxy benzenesulfonic acid, compound 1). Therefore we are suggesting that the values of the SIs and thetas observed in Tables 19 to 23 are influenced strongly by the low concentration of compound 1 in the environmental samples. This is illustrated in Table 24.

Table 19. Self-SI and Self-θ of Product Ion Spectra of m/z 241 Detected in Three Environmental Samples Obtained in 2014

Sample Location	Sample Time	Self SI	Self θ
Erie	07-03-2014	15.66±8.93	0.25±0.14
Daley	07-09-2014	10.23±5.86	0.15±0.09
Cicero	07-11-2014	16.48±8.97	0.24±0.12

Table 20. Self-SI and Self-θ of Product Ion Spectra of m/z 241 Detected in Three Environmental Samples Obtained in 2015

Sample Location	Sample Time	Self SI	Self θ
Erie	07-23-2015	6.00±4.18	0.075±0.046
Daley	07-25-2015	12.06±7.84	0.141±0.085
Cicero	07-24-2015	8.98±5.52	0.138±0.090

Table 21. Inter-SI and Inter-θ of Product Ion Spectra of m/z 241 Detected in Three Environmental Samples Obtained in 2014

Sample Pairs	Inter SI	Inter θ
Erie (07-03-14) vs. Daley (07-09-14)	21.20±7.80	0.32±0.12
Daley (07-09-14) vs. Cicero (07-11-14)	16.91±6.75	0.24±0.09
Erie (07-03-14) vs. Cicero (07-11-14)	18.75±8.92	0.29±0.13

Table 22. Inter-SI and Inter-θ of Product Ion Spectra of m/z 241 Detected in Three Environmental Samples Obtained in 2015

Sample Pairs	Inter SI	Inter θ
Erie (07-23-15) vs. Daley (07-25-15)	22.21±9.68	0.26±0.10
Daley (07-25-15) vs. Cicero (07-24-15)	18.84±9.57	0.23±0.10
Erie (07-23-15) vs. Cicero (07-24-15)	9.51±4.93	0.14±0.08

Table 23. Inter-SI and Inter-θ of Product Ion Spectra of m/z 241 Detected in Three Environmental Samples Obtained in 2014 and 2015

Sample Pairs	Self SI	Self θ
Erie (07-03-14) vs. Erie (07-23-15)	12.53±4.26	0.18±0.08
Daley (07-09-14) vs. Daley (07-25-15)	13.09±7.50	0.16±0.08
Cicero (07-11-14) vs. Cicero (07-24-15)	14.22±6.05	0.20±0.09

Changes in Similarity Index and Contrast Angle as a Function of Compound Concentration

Quantification of targeted compounds in complex matrices is normally carried out by isotope dilution and tandem mass spectrometry. When a suitable isotopically-labelled standard is unavailable, quantification is carried using an external calibration curve. Quantification by isotope dilution is not possible, but we can get a rough estimate of the concentration of compound 1 in the extracted environmental samples by comparing ion abundances with the standard samples used to generate the similarity indices. Quantification is always assessed in tandem MS methods by monitoring the abundance of the most abundant fragment ion in the product ion spectrum. For the spectra collected in this study, that is the m/z 205 ion. The average number of counts of m/z 205 in 3,5-dichloro-4-hydroxy benzenesulfonic acid's product ion spectra collected for the SI and theta analyses described in Tables 19 to 23 and standard deviations are compared to the self-SIs and thetas for each of the compounds. We estimate the concentration range of the dichlorosulfonic acid in the environmental samples using number of counts of m/z 205 as the following: Product ion m/z 205 of standard compound 1 with number of ion count of 146489 and its concentration is 1mg/ml. Product ion m/z 205 in environmental samples in Table 24 below ranges from 67 to 136. Therefore, the concentration of environmental samples can be estimated as the following equation:

$$\frac{146489}{1mg/ml} = \frac{67\sim136}{x\ mg/ml} \tag{6-7}$$

From above equation 6-7, the concentration of 3,5-dichloro 4-hydroxy benzenesulfonic acid is in the range of 4.6×10^{-4}~9.3×10^{-4} mg/ml. However, this is not the concentration in original environmental water sample taken from Chicago River, since it has been concentrated

by solid phase extraction procedure by a factor of 500ml/200µl = 2500 (Chapter 3). Therefore, the concentration of compound 1 in original water sample can be solved by dividing above range by 2500, that is 0.18~0.37 µg/L. We would expect that the self-SI and theta would decrease as ion abundance increase (more stable signal), however, this was not the case. We did not find any correlation between m/z 205 fragment ion abundance and the size of the SI.

Table 24. Average Number of Counts of m/z 205 in Product Ion Spectra of m/z 241 Derived from Environmental Samples Obtained in Both Year 2014 and 2015 and Their Self-SIs and Self-θ

Samples	Average Number of Counts of m/z 205	Self-SIs	Self-θ
Erie 07-03-2014	67±25	15.66±8.93	0.25±0.14
Daley 07-09-2014	105±22	10.23±5.86	0.15±0.09
Cicero 07-11-2014	76±25	16.48±8.97	0.24±0.12
Erie 07-23-2015	72±9	6.00±4.18	0.075±0.046
Daley 07-25-2015	136±35	12.06±7.84	0.141±0.085
Cicero 07-24-2015	86±19	8.98±5.52	0.138±0.090

In the next set of experiments, we investigated the change in self-SI and theta as a function of concentration of the dichloro 1,4-hydroxybenzene sulfonic acids. This investigation was carried out using direct infusion on an ion trap mass spectrometer because this arrangement could acquire more individual product ion spectra with this instrumental setup (versus an autosampler-based arrangement) over a shorter period of time.

Molecular weight and product ion mass spectra of the three dichloro-4-hydroxybenzene sulfonic acids were acquired with a ThermoFinnigan Advantage (San Jose, CA) LCQ ion trap mass spectrometer. The concentration of these sulfonic acids was varied and analyzed by direct infusion with a syringe pump at a flow rate of 50µl/minute. The same instrument parameter values were used to acquire all product ion spectra of the $(M-H)^-$ ions formed by the three sulfonic acid standards. The tandem mass spectra energy parameter was set to 35% of its

maximum value for fragmentation of all the molecular ions in this study. The concentrations were varied from 12.5 µg/ml to 250 µg/ml. For comparative purposes, eight individual product ion spectra were acquired by averaging thirty individual spectra for each compound acquired over the course of one minute. The capillary temperature was 200°C and the spray voltage was 4.5kV.

Table 25. Self-Similarity Indices and Spectra Contrast Angles as Function of Concentration for the Three Dichloro 1,4-hydroxybenzene Sulfonic Acid Standards Used in This Study

	Concentration(µg/ml)	Compound 1	Compound 2	Compound 3
Self SI	12.5	24.06±17.49	21.90±12.03	22.43±13.00
	25	16.59±9.62	15.16±7.03	16.52±10.19
	125	11.25±7.84	8.43±4.93	5.61±3.79
	250	11.29±8.01	7.52±3.79	7.07±4.75
Self θ	12.5	0.005±0.003	0.05±0.08	0.038±0.021
	25	0.004±0.003	0.04±0.02	0.020±0.011
	125	0.002±0.001	0.06±0.04	0.012±0.007
	250	0.003±0.002	0.04±0.03	0.009±0.004

The self-SIs and thetas for all the three 1,4-hydroxysulfonic acid isomers are presented in Table 25. The self-SIs are observed to increase by approximately a factor of three as the concentration of the sulfonic acid standards decrease a factor of 20. As the limit of detection is approached the variability of the relative abundance of any particular product ion is expected to increase which brings about more inconsistency in the product ion spectra being averaged for a similarity index or contrast angle calculation. The spectra contrast angle appears to increase less as concentration is lowered, since it is normalized by the length of each vector (spectrum) and a better method to distinguish spectra than similarity index [57], which is normalized by the sum of two relative abundances ($i+i_0$).

Comparisons of Similarity Indices and Contrast Angle Calculation using Statistical Test

In order to estimate the concentration of targeted compound necessary to make a distinction between two sets of spectra, we need to establish the concentration levels that would permit one to distinguish between the self-comparison with the inter-sample comparison. Here we would like to evaluate the effectiveness of using a t-test (comparison of two experimental means) to judge whether or not isomeric pollutants isolated from river water samples acquired from different locations and different times could be judged as having the same or different structures at low (less than 1µg/L) concentrations [56] likely to be found in the environment. Here we compare the Inter-SIs and thetas generated from the comparison of spectra derived from different samples to the self-SIs and thetas for individual samples. If a calculated $t_{statistics}$ is larger than a $t_{critical}$ that is dependent upon the number of degrees of freedom (DOF) at the 90% confidence level then the self-comparison is judged to be significantly smaller than the inter-comparison and the two compounds whose product ion spectra is being compared in the inter-comparison may be said to have different structures (or concentrations that are too small to be compared). If the converse is true, than the two compounds analyzed isolated from different water samples are verified as having the same structure. In this particular instance, again, we know, from cochromatography, that the structures of all the $[M-H]^-$ ions are consistent with that of 3,5-dichloro-4-hydroxy benzenesulfonic acid. Therefore we expect the self-SI and thetas to have the same value if the concentration is sufficient and there are no isobars coeluting with the compound that produces the m/z 241 ion that produce fragmentation that would contribute to observed the product ion spectra. T-test data comparing six different environmental samples are given in Tables 26 and 27.

Table 26. Student's (one-tailed) t Calculated from the Comparison of Product Ion Spectra Taken from the Different Sites along the Chicago River within One Day of Each Other (DOF= 14, $t_{critical}$ =1.345).

Sample Self-SI	Inter-SI Erie vs. Daley	Inter-SI Daley vs. Cicero	Inter-SI Erie vs. Cicero	Sample Self- θ	Inter-θ Erie vs. Daley	Inter-θ Daley vs. Cicero	Inter-θ Erie vs. Cicero
Erie (07-23-15)	39.01		8.18	Erie (07-23-15)	26.58		11.73
Daley (07-25-15)	17.00	10.77		Daley (07-25-15)	30.04	19.01	
Cicero (07-24-15)		20.64	0.66	Cicero (07-24-15)		9.65	1.38

Table 27. Student's (one-tailed) t Calculated from the Comparison of Product Ion Spectra Taken from the Same Site One Year Apart (DOF = 18, $t_{critical}$ = 1.330)

Sample	Erie 07-23-15 Self SI	Daley 07-25-15 Self SI	Cicero 07-24-15 Self SI	sample	Erie 07-23-15 self θ	Daley 07-25-15 self θ	Cicero 07-24-15 self θ
Erie 07-03-14 self SI	17.45			Erie 07-03-14 self θ	18.00		
Daley 07-09-14 self SI		3.46		Daley 07-09-14 self θ		2.11	
Cicero 07-11-14 self SI			14.56	Cicero 07-11-14 self θ			9.72

The data in Tables 26 and 27, suggest that the samples acquired at Cicero in the summer of 2015 and the sample acquired at Daley in 2014 may be sufficiently concentrated to carry out a definitive comparison of two different samples to determine whether or not the structures are the same or different.

Conclusion

Product ion spectra comparisons carried out with similarity indices or spectra contrast angle are useful to differentiate the structures of dichloro 1,4-hydroxybenzene sulfonic acid isomers that

possess common fragmentation pathways. Spectral contrast angle is also better than similarity index in distinguishing the standard isomers (inter-θ/self-θ ratios greater than inter-SI/self-SI ratios) in this application.

Product ion spectra acquired from m/z 241 ions isolated from different environmental water samples are strongly suggested to be 3,5-dichloro-4-hydroxy benzenesulfonic acid by cochromatography. The inter-θ/self-θ ratio is almost the same as the Inter-SI/self-SI ratio from the comparison of product ion spectra of six environmental samples. Spectral contrast angle and similarity index do not make a difference in the comparison of product ion spectra acquired for environmental samples as they are hoped to, since spectral contrast angle should allow us to distinguish product ion spectra that have very subtle differences [42]. The comparison of product ion spectra acquired from six different environmental samples suggests that in most cases, the concentration of the 3,5-dichloro isomer are too small to carry out meaningful sample to sample comparisons for the purpose of assessing structural similarity.

Statistical analysis of the product ion spectra suggests that the minimum concentration necessary to carry out meaningful comparisons of product ion spectra acquired from two samples, Cicero (07-24-15) sample with concentration of 0.23µg/L and Daley (07-09-14) sample with concentration of 0.29µg/L.

APPENDIX A

PRECURSOR IONS FOUND CONTAIN AT LEAST ONE CHLORINE IN EXTRACTED STICKNEY WASTE WATER EFFLUENT IN 2012 AND 2013

Sample Tested in 2012		Sample Tested in 2013	
Precursor ion m/z	Retention Time	Precursor ion m/z	Retention Time
203.0	17.8	206	17.6
208.7	7.7	211.1	17.4
211.0	17.1	217	20.0
217.1	24.1	222.2	13.4
220.0	13.3	228.2	20.4
230.9	19.6	231.2	19.7
228.0	15.6	237.1	12.2
233.0	24.3	245/247/249	21.2
240.9/242.9	5.05	251	19.0
244.9/246.9/248.9	22.0	257	9.78
251.1	19.6	258.1	23.1
253.0	26.0	262.8	6.81
256.8	9.65	262.7	13.5
258.1	25.8	265	20.1
261/263	19.2	266	18.0
265	20.5	285.9	23.2
267.1	26.2	287/289	26.6
283.1	28.6	297.1	22.7
285.1	17.3	307.1	7.73
286.0	25.3	313	21.1
287.0/289.0	30.0	330	23.4
296.9	23.9	335.1	19.4
300.1	27.9	345.8	17.3
301.0/303.0	18.5	367/369/371	27.0
317.0	21.0	395/397/399	7.70
326.8	27.1	410.0	23.7
330.1	27.1	421.2	16.9
351	21.2	431.1/433.1/435.1	7.79
359.1/361.1	7.5	441.1/443.1/445.1	7.74
367/369/371	30.4	487.4	12.4
385/387/389/391	28.7		
395/397/399	7.5		
410.1	27.2		
431/433/435	7.4		
437	16.3		
441/443	7.5		
479	21.9		
493/495/497	25.3		
517/519/521	20.8		
591.2	7.4		

APPENDIX B

PRECURSOR IONS FOUND WITH AT LEAST ONE CHLORINE IN LAKE MICHIGAN SAMPLE NEAR BUCKINGHAM FOUNTAIN

May 9th 2013		June 11th 2013 Sample 1		June 11th 2013 Sample 2	
Retention time (min)	m/z	Retention time (min)	m/z	Retention time (min)	m/z
30.0	287/289/291	28.0	367/369	21.5	545/547
30.0	242.9	24.2	315	22.5	333
30.1	284.9	22.5	333	22.5	401
30.1	245	22.5	401	19.4	387
30.5	277/279	25.9	389/391	20.8	201
22.4	333.2	19.7	421	21.6	299
30.5	292.9	25.8	399/401	30.3	245
19.3	330	23.7	299	30.3	287
21.9	331.2	20.2	331	30.4	243
25.8	399/401	20.1	377	21.4	331
31.6	313	29.4	417/419/421	7.74	395
23.6	273	21.4	383	30.4	277/279
21.0	332	19.4	354	25.9	399/401/403
18.4	327	23.0	303	31.5	200
31.4	200	21.4	305	29.6	427
19.3	341	30.2	289	20.1	377
30.3	207	18.2	301	29.5	417/419
12.1	488	7.75	395	25.9	390
25.8	401/403	21.4	591	30.5	290
19.7	421	14.1	285	20.9	227
20.4	228	21.5	349	9.28	209
19.4	263/265	17.9	376	21.5	305
16.8	344	20.0	257	29.6	429
15.9	299/301/303	20.2	360	30.2	285
31.6	315	30.1	287	21.2	313
14.2	285	19.9	427	17.4	219
20.1	377	17.8	340	29.5	419
22.2	485	23.6	361	20.7	223
17.8	206	31.3	200	19.2	253
		22.0	305	13.4	329
		18.7	255	19.4	354
		19.3	341	19.8	213
		19.9	429	14.2	287/289
		19.2	245	19.4	367
		20.6	307	19.3	256
		17.8	380	13.5	258
		22.0	219	17.4	313/315
		30.4	281	20.6	227
		13.9	221	19.9	357
		13.6	220	21.4	591

				17.8	340
				21.4	205
				17.8	344
				9.29	210
				17.7	206

APPENDIX C

PRECURSOR IONS FOUND IN SEVEN LOCATIONS OF THE CHICAGO RIVER

Madison	05-09-2013	Madison	05-30-2013 sample 1	Madison	05-30-2013 sample 2
Retention time (min)	m/z	Retention time (min)	m/z	Retention time (min)	m/z
27.91	366.8	27.32	366.9	30.53	287/289
29.45	427.4	24.13	410	27.89	315
25.05	328.6	14.46	226.9	30.46	242.9
25.16	330.7	21.19	245	17.32	366.8
31.46	312.8	19.77	250	7.81	395
25.81	399.3	6.43	240.9	25.57	313.1
25.96	543.4	13.53	220.1	25.45	244.9
7.84	441	17.31	210.6	30.8	277/279
17.62	205.9	7.51	228	17.88	211.1
19.85	341	21.43	277.2	13.72	220
18.43	327.3	19.24	263	14.86	226.9
25.82	401.1	21.42	591.1	27.52	223
29.84	419	15.13	233.2	16.28	370.9
14.52	205	21.46	517/519	7.52	227.8
25.84	403	21.7	217.2	19.52	391
14.55	227	29.77	426.9	21.92	264.7
18.36	231	22.37	253.2	18.85	258.1
18.57	233	7.76	440.8	15.58	233.1
25.97	391	20.12	278.8	20.1	213
25.84	545.2	13.35	219.2	19.81	250
20.10	355.1	17.35	202.9	17.38	219
13.52	220	21.94	265	17.39	203
25.82	541.3	14.56	205	19.26	286.2
25.90	533.2	7.47	202.1	19.73	230.8
8.86	336.8	25.91	543.2	19.48	291.1
31.29	200	18.91	235/237	19.1	236.6
16.00	227.9	19.13	390.4	16.92	421.2
12.13	487.2	7.71	507	7.59	202.1
19.38	335.1	16.88	421.2	17.73	206
19.74	421.3			17.37	357/359
19.04	339			19.8	293.8
23.68	371.3			7.5	230
16.86	209.1			26.36	217.2
21.45	405			19.64	260.1
				18.58	215.1
				14.66	204.6

West River Park	June 27th, 2013
Retention Time (min)	m/z
25.52	245
22.46	333
21.90	265
22.29	289/291
13.53	220
17.74	211
19.61	277
21.05	287
19.80	240
17.66	206
31.40	200
30.60	331
8.68	337
25.20	317
21.55	378
18.58	331
25.23	315
20.11	377
21.54	274/276
21.42	383
29.33	341
22.24	335
16.82	342
17.23	482
21.60	405
21.07	313
21.36	335
21.10	545
21.54	228/230
25.2	273
22.48	401
24.66	317
19.91	427
25.82	313
24.60	321
22.57	411
18.35	413
19.42	387
19.79	240
19.71	231
18.21	204
14.98	258

16.88	254
21.39	591
21.28	299
20.30	235
17.12	257
21.92	270

Weed Street	June 27th, 2013
Retention time (min)	m/z
21.93	245
15.55	227
17.44	211
14.41	227
22.66	367
22.41	399/401
21.67	451
21.60	381
22.42	435/437
22.21	365
13.45	220
31.33	317
20.78	383
26.58	329
22.14	250
22.19	553
22.26	441
21.85	265
22.84	217
16.44	231
21.84	210
16.67	309
21.53	591
21.49	310
19.54	277
25.16	237
20.11	261
24.86	330
11.62	275
21.08	577
21.22	274/276
14.91	257
22.43	333
25.50	419
13.00	258
19.69	242
21.05	287
16.77	254
19.76	240
19.82	235
9.01	337
16.42	231
17.86	204

19.28	286
1.83	200
18.20	233
16.80	342
25.42	410
14.88	293
21.17	276
17.56	206

Daley Park	July 11th, 2013
Retention Time (min)	m/z
29.22	330
29.04	286
19.61	367
7.59	395/397/399
22.45	333
29.18	410
23.20	297
19.65	287
23.96	517/519
27.48	245
21.98	443/445
7.72	441
19.58	385/387
20.86	346
22.08	453
7.73	431/433
20.58	293/295
6.48	241
19.95	421
16.37	437
20.85	426
13.78	220/222
16.95	421
21.00	423
18.06	211
21.08	313
19.56	343
19.74	250
20.10	279
19.17	259
31.56	200
16.61	219
19.10	239
22.63	253
15.04	227
21.89	265
16.59	308
20.43	228

Willow Spring	July 11th 2013
Retention time (min)	m/z
28.98	330
26.89	245
24.24	299
7.75	395
28.75	217
29.25	286
22.81	577
22.66	563
22.69	253
22.38	289
21.15	251
26.86	371
24.09	410
24.33	286
22.83	291
26.82	367
25.01	271
16.82	228
17.15	231
21.44	591
22.33	333
16.91	421
10.63	257
16.37	437
21.81	269

Erie Street	Aug 14th, 2013
Retention time (min)	m/z
28.28	245
6.38	241
30.71	330
24.80	519/521
30.60	410
24.98	265
21.17	346
7.64	441/443
20.82	225
30.59	217
18.25	209
22.50	333
17.43	203
7.87	431/433
16.68	255
16.27	437
19.08	237
17.01	421
18.13	211
18.98	229
16.58	219
19.06	227
20.87	223
19.55	387
13.51	279
18.98	353
21.45	591
22.86	578
19.56	307

Cicero Ave	Aug 21st, 2013
Retention time (min)	m/z
29.80	330
7.75	395
20.22	367/369
30.08	410
20.87	223/225
20.61	289
19.40	286
19.58	387
30.20	286
6.53	241
7.62	359
7.75	433
18.81	211
19.17	237
16.92	437
17.24	353
16.98	421
21.11	313
18.28	209
19.52	261
13.78	220
19.85	250
19.66	277
19.07	229
7.82	587

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